THE POPULATION GENETICS OF TWO TEMPERATE RAINFOREST TREES, *LAGAROSTROBOS FRANKLINII* (Hook f.) Quinn (HUON PINE), AND *ATHEROSPERMA MOSCHATUM* Labill. (SASSAFRAS).

BY
ALISON SHAPCOTT

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DECLARATION :

This thesis contains no material which has been accepted for the award of any other degree or diploma in any University, and contains no copy or paraphrase of material previously written by another person, except where due reference is made in the text.

A. Shapcott.

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ABSTRACT :

The population genetics of two temperate rainforest tree species endemic to south eastern Australia were studied. Both species are long-lived and members of ancient families. There are parallels between the two species even though one was a gymnosperm and the other an angiosperm. For example, both species reproduce both vegetatively and sexually. *Lagarostrobos franklinii* (Huon pine) (Podocarpaceae) is mostly dioecious and wind pollinated, while *Atherosperma moschatum* (sassafras) (Monimiaceae), is monoecious or dioecious and insect pollinated. Both have potential for long distance seed dispersal, *L. franklinii* by water and *A. moschatum* by wind.

The population genetics of both species was studied from stands throughout their geographic range using isozyme analysis. Most genetic diversity was found within rather than among sites. Genetic diversity among sites was low but generally consistent with expectations for each species (Hamrick and Godt 1979). *Atherosperma moschatum* had much more diversity among sites than Huon pine, with its mainland sites differentiating significantly from its Tasmanian ones. In Huon pine, most differentiation was found in isolated sites. Diversity within sites was also low in Huon pine but was much greater in sassafras. The structure of genotypes within stands was examined using spatial autocorrelation. In both species trees of like genotypes were found to be clustered at short distances. This genetic substructuring was found regardless of population size, density, distance from other stands, level of inbreeding, history, etc. Most sites deviated from Hardy-Weinberg expectations with deficiencies of heterozygotes, and high levels of allelic fixation, and were effectively inbred.

The size structure and floristics within stands were investigated and used to assist in the interpretation of the patterns of genetic variation, inbreeding and stand dynamics found in each species. There was much variation in size structures and regeneration modes between sites in both species and neither appeared to require large scale disturbances for regeneration. The two species varied in the relationships between site environmental/ecological similarity and genetic similarity. In both species there was as much diversity in genetic variability and size structure in small isolated stands as there was in stands within larger assemblages.

The proportion of trees contributing to the reproductive population, as well as the proportion of each gender type within that population, were estimated for Huon pine stands. On average thirty percent of Huon pine trees greater than one metre tall were reproductively active in the mast year recorded, and overall there were equal proportions of male and female trees. The relationships between reproduction and gender expression, with size structure, density, floristics, inbreeding and genotypes were investigated. Stands were also compared to identify if there were geographical or climatic trends in the distribution of these characteristics. Reproduction was found to increase with increasing tree size and also with more open canopies. Sites with similar proportions of females were found to also have similar species compositions. The distribution of reproductive trees and gender types within stands was investigated using spatial autocorrelation. The results were compared with genotypic distributions within the same stands. Although there was no direct correlation between gender type and genotype, both genotype and gender type were clustered at the same spatial scale, suggesting that such clustering may have a strong vegetative component.

Huon pine seed production was estimated at one site and seed dispersal was investigated. Very large quantities of seed were shed. Seed dispersal laterally was negligible, but potential for dispersal down water courses was great as it stayed afloat for extended periods. Huon pine seed germination was investigated both in the field and under experimental conditions. Germination generally was slow, and with a low success rate. However seed in the field germinated at particular daylengths (regardless of temperature) in two consecutive seasons.

Both species showed evidence that vegetative reproduction and localised pollen and seed dispersal have led to the development of family clusters, leading to inbreeding, and local fixation of allelic proportions. However infrequent long distance gene flow has probably reduced population differentiation. The population viability of each species was discussed.

GENERAL INTRODUCTION :

The conservation and maintenance of biodiversity implies not only the maintenance of species and ecosystem diversity, but the diversity within species (Hopper and Coates 1990, Lacy 1988). The diversity within species may give flexibility to adapt to changing conditions and to survive major perturbations such as disease, or may be the source for future new species (Ellstrand and Antonovics 1985). In this era of increased domestication and genetic manipulation of species, it is also important that the range of genetic variation in natural populations be conserved and understood (Brown 1978, Hopper and Coates 1990). As there is not time to study each species, conserving and understanding the dynamics of, for example, major canopy or habitat forming species may ensure survival of much of their dependent biota (Boyce 1992).

In order to determine the viability of a species, it is important to understand how the species functions at many levels, from individuals, to stands and populations and how each level interacts (Boyce 1992). Viable populations can adapt to changing conditions and recover or re-establish after major perturbations (Shaffer 1987, Boyce 1992). Information that may assist in the determination of species/population viability includes its regeneration patterns and requirements, and age structures (Boyce 1992, Lande 1988). Regeneration is influenced by ability to reproduce, the relative importance of sexual and vegetative reproduction and by seed germination requirements (Lande 1988, Harman and Franklin 1989). The viability and survival of offspring may depend on their genetic makeup, especially if the species is under strong selection from a changing environment (Brown 1989, Coates 1992, Ennos 1989, Muona 1989). Thus a species' potential for seed dispersal, its germination and establishment requirements and its genetics will determine to a large extent its ability to expand its population, re-establish itself following perturbations or colonise new sites (Ellstrand 1992, Schupp *et al.* 1989). In many cases the viability of a species will also depend on its ability to survive in small, subdivided or fragmented populations (Lynch and Gabriel 1990, Krusche and Geburek 1991, Hopper and Coates 1990, Lande and Barrowclough 1987). Many factors may determine this, such as the relative importance of sexual and vegetative reproduction (Ellstrand and Antonovics 1985), the degree of inbreeding, whether a species is prone to inbreeding depression (Schemske and Lande 1985), and the amount of genetic diversity and the potential for its maintenance (Levin *et al.* 1970). Genetic diversity may be determined by the breeding system, pollination mechanism, dispersal characteristics

and the germination and regeneration requirements of a species and its generation time (Hamrick and Godt 1989, Levin 1988).

It is therefore obvious that population genetics and ecology are intertwined and essential for an understanding of species/population viability. In particular an understanding of the ecology of a species, especially as it relates to population dynamics, will enhance the interpretation of population genetic results. Several authors have called for more integration of ecology, demography and genetics (Levin 1978, Lande 1988, Hamrick *et al.* 1979, Antonovics and Levin 1980, Antonovics *et al.* 1988, Boyce 1992). However, there are still relatively few studies on plant species, which test for associations between ecological or demographic data and genetic data (e.g. Xie *et al.* 1992, Finns and Libby 1982, Linhart *et al.* 1979, Govindarju and Dancik 1987). Some studies have tested one particular set of variables, such as density with inbreeding (Antonovics and Levin 1980, Linhart *et al.* 1981, Murawski and Hamrick 1991, Brown 1989), but most references to ecological determinants of genetic structure are speculative. Case studies of a variety of types of species not only enable detailed understanding of those particular species, but they build up more complete pictures of real as opposed to theoretical populations (Antonovics and Levin 1980). These serve to support or challenge theories which have developed to explain or predict aspects of species' or population functioning (Antonovics and Levin 1980).

An understanding of the regeneration requirements of species is fundamental for conservation management (Boyce 1992, Shaffer 1987). Observations of regeneration patterns in natural populations assists in understanding the requirements for, or likelihood of, persistence of these populations (Boyce 1992, Whitmore 1989). Many species do not successfully regenerate populations on a particular site as their very presence changes the environment such that it is no longer suitable for their establishment (Whitmore 1987, Spies and Franklin 1989, Ogden 1985). Climax species would be expected to be able to regenerate beneath their own canopies (Whitmore 1989). However, many rainforest species require small scale disturbances for re-establishment (Whitmore 1989, Spies and Franklin 1989, Canham 1989). Some species rely on producing large numbers of offspring each season, each with a low chance of survival (R species), while others produce fewer offspring, more infrequently, each with a greater chance of success (K species) (MacArthur and Wilson 1967).

The diversity within a species may affect its potential for adaptation, its fitness, and its ability to survive perturbations, such as caused by disease or predation, and hence,

species' viability (Muona 1989, Burdon and Jarosz 1989, Ellstrand 1992, Lande 1988). Inbreeding, clonal growth and poor dispersal potential, are generally thought to lead to greater diversity among populations, but lower total diversity (Hamrick and Godt 1989, Ellstrand and Roose 1987, Brown 1989). Characteristics which are likely to allow greater gene flow between populations, such as long distance seed dispersal and wind pollination, will tend to decrease the diversity among populations, but increase diversity within populations (Hamrick and Godt 1989). Alternatively selection for particular traits can counter the effects of gene flow and act to promote localised differentiation (Antonovics and Bradshaw 1970, Linhart *et al.* 1979, Wright 1978).

Although the need to conserve genetic diversity is often stressed, there have been few baseline studies that map and describe the amounts and patterns of genetic variation or the breeding systems of rainforest plants (e.g. Murawski and Hamrick 1991, O'Malley *et al.* 1988, Eguiarte *et al.* 1992, Gan *et al.* 1981) . The ecology of temperate rainforest trees species has been studied by many authors (e.g. Read 1989, Ogden 1985, Wardle 1984, Karlin Arroyo *et al.* 1993), but there are few studies on their population genetics (e.g. Hawkins and Sweet 1989, Haase 1992 a&b, Billington 1991). Hamrick and Godt (1989) have found some general trends in the patterns and amounts of genetic diversity between plants within broad categories of lifehistory traits and ecology. However, unbalanced representation of particular groups within some categories may have lead to some distortion of results. For example, most of the gymnosperms and long-lived species surveyed would have been northern hemisphere species, especially the Coniferaceae (Muona 1989). Southern hemisphere gymnosperms are dominated by the Podocarpaceae, which differ from the Coniferaceae in many ecological and lifehistory aspects. Therefore generalisations about genetic structure and diversity based on northern hemisphere gymnosperms, cannot be assumed to hold for southern hemisphere gymnosperms trees. To date, the results from some New Zealand studies (Hasse 1992a &b, Hawkins and Sweet 1989, Billington 1991), have indicated lower genetic diversity in several gymnosperms and in *Nothofagus truncata* than would be expected on the basis of Hamrick and Godt's (1989) work. There have also been relatively few studies of long-lived angiosperm trees (Muona 1989). Therefore studies of long-lived temperate gymnosperm and angiosperm rainforest trees may help clarify whether southern species conform generally to the trends identified by Hamrick and Godt (1989). Fewer population genetic studies have been undertaken on older more stable species than on more recently evolved species (Hamrick and Godt 1989). Most Tasmanian temperate rainforest tree species belong to old families and genera (Hill 1990). An

that this is not necessarily the case (Moran and Hopper 1987), and heterozygotes may be selected preferentially (e.g. Coates 1992, Muona 1989). The viability of small populations may depend on proximity to, and gene flow between, neighbouring populations, the effects of inbreeding or selection against it (Lande and Barrowclough 1987, Gregorious and Roberds 1986, Whitlock and MacCauley 1990, Barrett and Husband 1989 Coates 1992). However, there are relatively few studies published which compare breeding and variability in both large and small or subdivided populations of the same species (e.g. Van Treuren *et al.* 1991, Husband and Barrett 1991, Moran and Hopper 1987, Billington 1991). The study of populations which have long existed in a small isolated state, compared to their counterparts with larger populations, may give indication as to some of the critical factors required for viability of small populations and the effects of population fragmentation.

An understanding of the population functioning of a species, may lead to contributions to the clarification of more fundamental questions regarding evolution and survival generally. For example, many plants reproduce both sexually and vegetatively. There is much debate as to how sex evolved as well as to why and how it is maintained by species/organisms (Leigh 1970, Lloyd 1980, Koella 1988, Maynard-Smith 1978, Bell 1985). The rewards of sexual reproduction are said to include increased variability within species, since recombination allows interchanging of gene complexes, and heterozygosity allows variant alleles to be carried (Goodnight 1988, Farris and Mitton 1984). It has also been suggested that sexual reproduction is favoured for its maintenance of minority genotypes (Ellstrand and Antonovics 1985). A variable population is less likely to react uniformly to particular environmental pressures, such as disease (Antonovics *et al.* 1988). The costs of sex however, may include the reduction of specialised adaptation to particular environments due to the effects of recombination (Jain 1976, Antonovics *et al.* 1988), whereas vegetative reproduction allows the continuation and spread of successful genotypes and gene complexes (Ellstrand and Antonovics 1985). In species with options for both sexual and vegetative reproduction, variability in the balance between the expression of vegetative and sexual reproduction among populations may provide some insights as to the forces favouring one or the other .

Population density may have an effect on the amount of inbreeding and the relative proportions of vegetatively or sexually derived offspring (Antonovics and Levin 1980). The amount of inbreeding may also be related to the proportion of reproductively active trees, and the gender balance (Jain 1976, Lande and Barrowclough 1987). Density effects promoting inbreeding may be countered in

some species by density dependent selection, acting, for example, against inbred individuals, favouring sexually derived offspring or by favouring heterozygotes (Antonovics and Levin 1980, Muona 1989). The density of populations may be principally affected by site factors such as soil fertility, but may also be determined by biotic factors leading to density dependent self-thinning (Antonovics and Levin 1980).

In this study two species were studied in order to develop an understanding of their population functioning and requirements for their future long-term viability. They share many features. For example, both of the species are members of old southern hemisphere families and belong to species-poor genera. *Lagarostrobos franklinii* (Huon pine), belongs to the Podocarpaceae (Quinn 1982). At present there are two *Lagarostrobos* species. However, recent taxonomic revision may divide the genus (Molloy pers. com). *Atherosperma moschatum* (sassafras) is the sole member of its genus, and is part of the primitive angiosperm family Monimiaceae (Foreman 1984, Schodde 1969). Both species are dominant canopy trees with shade tolerant seedlings (Read 1985). Huon pine is presently endemic to south western Tasmania. *Atherosperma moschatum* occurs predominantly in Tasmania, but has populations extending to mainland Australia with outliers as far north as central New South Wales (Hill *et al.* 1988). Both species often occur in small stands as well as subdivided and disjunct populations. Many small stands are likely to have been stable for considerable periods of time (Neyland 1991, Anker *et al.* 1993). Both species have long lifespans and are likely to have been affected by repeated population contractions, bottlenecks and founder events due to past climatic change (Hill 1990, MacPhail 1979). Huon pine is mostly dioecious while *A. moschatum* is mostly monoecious (Schodde 1969). Huon pine is wind pollinated, as is typical of gymnosperms, while *A. moschatum* is insect pollinated, which is more typical of angiosperms. Both species also reproduce vegetatively. Both have potential for long distance seed dispersal, Huon pine by water, *A. moschatum* by wind.

Thus the two species investigated in this study have many ecological and lifehistory characteristics in common, but differ in some which are expected to affect the amount and distribution of their genetic diversity (Hamrick and Godt 1989). Are the similarities and differences between the population genetics of these species accounted for by these similarities and differences? Are general genetic expectations based on lifehistory traits and ecology met in these species? If there is no strong selection, populations in closer proximity to one another would be expected to be more genetically similar, than the more isolated and geographically distant populations due to isolation by distance leading to random drift (Sokal and

Wartenberg 1983, Wright 1978, Moran and Hopper 1983). Particular genotypic combinations may be favoured selectively in particular environments, such as particular climatic or fertility regimes (Bradshaw 1984, Barrett and Husband 1989, Antonovics *et al.* 1988). Thus genotypes may be correlated with specific environmental/ecological factors which may indicate evidence of selection, or genotypes may show geographical associations. The history of populations may also affect population genetics (Hopper and Coates 1990, Hawkins and Sweet 1989, Boileau *et al.* 1992). For example a history of colonisation, or a history of bottlenecks, may have impacts on a species present overall genetic diversity, diversity within populations, and the amount and effects of inbreeding (Nei *et al.* 1975, Schwaegerle and Schaal 1979, Husband and Barrett 1989, Boileau *et al.* 1992). Understanding the population genetics of small and subdivided populations especially in comparison to larger ones of the same species, as is undertaken in this study, enables a better assessment of the potential genetic viability of present small populations (Moran and Hopper 1987, Coates 1988, Billington 1991). When knowledge of the dispersal and regeneration properties of these species are incorporated with such genetic analysis, as was done in this study, a more realistic assessment of the present and future requirements for the viability of such populations may be made (Boyce 1992, Lande and Barrowclough 1987).

More specifically, the demographic viability of present populations was assessed by investigation of regeneration patterns, and some factors affecting regeneration success such as for Huon pine, germination requirements and success. In particular the question was asked, is there evidence that populations of these species are self-sustaining, or that they require major perturbations for regeneration (Whitmore 1989, Ogden 1985)? Regeneration in some species is primarily by vegetative means, while in others it is purely from seed (Bradshaw 1972). In species, such as these which are capable of both modes of regeneration different strategies may be favoured in different environments and may have an impact on the regeneration patterns and stand structures (Barrett and Husband 1989, Bradshaw 1972, Freeman *et al.* 1976, Moran and Hopper 1983). Dispersal characteristics, reproductive potential and regeneration requirements may affect a species' ability to recolonise after major perturbations, and therefore may determine the vulnerability of a species to local population extinction (Lande and Barrowclough 1987, Boyce 1992).

This study will gather base line data on the population genetics and population biology and structure of two temperate rainforest trees (Huon pine and *Atherosperma moschatum*)which share many common features of their biology, lifehistory traits and ecology, but which differ significantly in others. It will be determined whether

these similarities and differences are reflected in their population genetics, and whether the genetics of either species are consistent with expectations for species with similar lifehistory characteristics (Hamrick and Godt 1989). It will be assessed whether patterns in genetic expression are correlated with environmental or ecological variables, which may be expected to reflect differential selection pressures, such as climate or species composition. Furthermore, it is aimed to see if variation in factors affecting population dynamics, such as stand structure, stand density, size of reproductive population, or sex ratios are reflected in variations in population genetic characteristics, such as the degree of inbreeding. The study will investigate the present regeneration patterns and estimate some of the regeneration requirements of each species. In addition to the specific information gathered in this study, available information on the ecology and biology of each species is utilised to assess some aspects determining the viability of populations of these two species and especially to assess if small populations of these species are likely to be viable in both the short and long-term.

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CHAPTER 1 :

Seedfall in Huon Pine and its dispersal and establishment.

INTRODUCTION

Huon pine (*Lagarostrobos franklinii* (Hook f.) Quinn) is a Tasmanian endemic conifer with restricted distribution in the south-western part of the state. It is highly prized for its timber and has been subject to logging for 180 years (Gibson 1986). Huon pine is extremely slow growing but long-lived (Francey *et al.* 1984). Its population numbers have been severely reduced due to human activities (Gibson 1986). An understanding of the reproductive, dispersal and establishment capabilities of Huon pine will contribute to assessments of its requirements for long-term viability and hence its conservation.

The extant distribution of most Huon pine stands close to rivers, creeks or drainage lines, where the species often forms a thin fringe of trees on the banks (Peterson 1990), has led Millington *et al.* (1979) and Gibson (1986) to suggest that lateral dispersal of Huon pine is poor and that most dispersal is down rivers or moving water bodies. Where Huon pine occurs over wider areas, for example on river flats, slopes and in limestone sink holes (e.g. Pedley *et al.* 1980), it is associated with moist, often boggy conditions and is found in locally fire protected areas of high rainfall (Gibson 1988). Some evidence of expansion of Huon pine stands into previously fired areas has been recorded by Davies (1983) and Gibson (1986).

Huon pine is known to reproduce vegetatively as well as sexually (Millington *et al.* 1979). The vegetative reproduction observed offered only limited dispersal opportunities since propagules are limited by the extent of the root system or the length of the fallen trunk of the parent tree. Davies (1983) and Pedley *et al.* (1980) have speculated that fallen twigs may lodge, take root and shoot and that these twigs may be dispersed by water, particularly by floods to a suitable site. However, these seem to be rare events and most regrowth along rivers appears to result either from local vegetative reproduction or from seedlings.

Huon pine is mostly dioecious (Quinn 1982) and its sexual reproductive activity is commonly thought to be episodic with years of synchronised high seed production (mast years) occurring approximately every 5-7 years. Such a mast year occurred in

the 1988/89 season. This season also coincided with high reproductive activity in many other local rainforest species.

Many authors (e.g. Read and Hill 1983, Harman and Franklin 1989) have suggested that logs and trunks may form major sites for seedling establishment. The lack of such suitable germination sites may consequently limit establishment possibilities.

This chapter investigates the pattern and timing of seed fall in a mast year in Huon pine. In addition several vectors of seed dispersal are investigated including lateral dispersal by wind and by green rosellas (*Platycercus caledonicus*) and the potential for long distance dispersal by water. Optimum conditions for germination were investigated in the glasshouse. Field observations were compiled to determine the relative importance of habitat for seedling establishment. Finally, downstream colonisation by Huon pine at Condominium Creek was recorded and an approximate timeframe for this process determined.

METHODS

Seedfall and Dispersal

A study site was established at Riveaux Creek (Lat.43°11' Long. 146°40') near the Picton River, where a number of longer term experiments were established and monitored. Seed traps were erected under heavily laden female cone-bearing trees, to monitor the timing and quantity of seedfall. The traps were of two types, but both collected over a $1m^2$ area. The first type was made of galvanised iron with a circular-mouthed funnel. The second type was made of sail cloth and PVC piping with a square-mouthed funnel. Both had a wire-mesh based bucket attached to the base of the funnel to collect seed and an open stocking leg placed in the bucket to serve as a filter and collection bag. The traps were mounted on wooden stakes. The seed was removed approximately monthly. The stocking contents were air dried prior to sieving to remove extraneous material. Each sieved sample was weighed. A weighed sub-sample of 1-2g was then sorted and the seed counted; undersized seed was removed at this stage. Quantities of seed collected were extrapolated from these values. Seed traps were also established at Teepookana (Long. 145°21' Lat. 42°14'). At both sites traps were laid away from trees to estimate lateral seed dispersal. These seed traps were located at one tree height (15-20m) and in the case of Teepookana also at three tree heights (Figure 1.1) from the source tree. The timing of cone development and the dates on which different developmental stages occurred were recorded on visits to Riveaux Creek.

Lateral seed dispersal was further investigated by soil/litter samples. These were taken from two parallel transects from tree (1) at Riveaux Creek in November 1989 (Figure 1.1a). Quadrats 24cm x 24cm were marked and scraped bare of surface soil, litter and moss, which was collected for analysis. These samples were later flushed through a sieve with water, air dried, resieved and the number of Huon pine seeds estimated as for the seed traps. Quadrats were located at mid-canopy, at the edge of the canopy and at 4 metre intervals up to 12 metres from the canopy (Figure 1.1a). Seed numbers were also used to estimate the amount of seed still on the ground several months after seedfall.

Green rosellas (*Platycercus caledonicus*) were observed feeding on Huon pine at Riveaux Creek. The timing and duration of feeding behaviour and the trees visited were recorded using a combination of direct observation and ground evidence, consisting of broken terminal shoots.

Seed Floatation

An experiment was carried out to investigate the length of time for which Huon pine seeds can float. There were three replicates each of one thousand seeds collected from tree (1) at Riveaux Creek, which were placed in a jar half filled with tap water. A porous cloth covered the opening of each jar. The seeds were swirled approximately once per day and every few days the water was drained through the cloth and the jars refilled. These procedures ensured that the seeds were not floating due to surface tension and prevented algal development. The number of floating and submerged seeds was counted regularly. Following the experiment samples of seeds that remained floating, submerged seeds and air dried seeds of a similar age, were subjected to a squash test in order to monitor embryo deterioration. Samples of submerged and air dried seed were sown to test germination.

Germination and Establishment

Fresh seed collected from a single tree at Riveaux Creek (tree 1) was used to set up a combination of germination experiments. Four replicates of 100 seeds were used for each combination of treatments. Three temperature treatments were set up as follows: 10°C, 20°C and 10-25°C diurnal fluctuation. Temperature treatments were conducted in growth cabinets with a 14 hour photo-period. Germination was also examined under glasshouse conditions with no artificial light. Seven physical treatments were carried out under the four temperature regimes. They were:

- 'untreated',
- 'soaked' in tap water for one week prior to sowing,
- 'damaged' by pricking with a needle,
- 'stratified' for '30' days,
- 'stratified' for '100' days,
- sown in 'sand', and
- sown in river 'soil'.

All seeds were sown in clear plastic containers with lids. Except for the 'sand' and 'soil' treatments, they were sown onto filter paper overlying a layer of cotton wool. All seeds were dusted with Thiram® fungicide prior to planting. Seeds were kept moist by regular watering with tap water. The experiment ran from April 1989 until March 1990, and was scored fortnightly until week 22 and then as indicated (Figure 1.2). At each scoring the seeds were recorded in three categories:

1. open seed, i.e., the seed coat had split open,
2. emergence of root or hypocotyl from the seed, and
3. cotyledons present and expanded.

There was some embryo mortality. The results of stages (2) and (3) were pooled for some analyses (Figure 1.5), since these represented an assessment of successful germination.

One metre square plots were set up at Riveaux Creek to monitor the timing of seed germination in the field and the effects of animal browsing on seedling populations. Two exclosures were erected and consisted of a $1m^3$ chickenwire construction supported by metal star pickets. The wire was tacked to the ground surrounding each structure to prevent animals burrowing in. Beside each exclosure was a $1m^2$ unprotected plot. One pair of plots was located near a seed trap (No.2) at the edge of the stand and the other in an area well within the stand and under a fecund female tree. These were monitored regularly and seedlings were mapped on a grid. The site was also generally monitored for the emergence of seedlings.

The number of seedlings established on logs or trunks (including logs or trunks of other species) and ground (soil, moss and rock) was recorded in the field from nineteen sites (see Chapter 2). At each site belt transects, which encompassed much of the stand, were surveyed, the number location and substrate of seedling were recorded. Isolated individual stems less than one metre tall were classified as seedlings, but it was not possible in all cases to determine if these were actual seedlings or were vegetative growth from roots or trunks. Major excavations were not usually possible or desirable. As a consequence seedling numbers may have been overestimated.

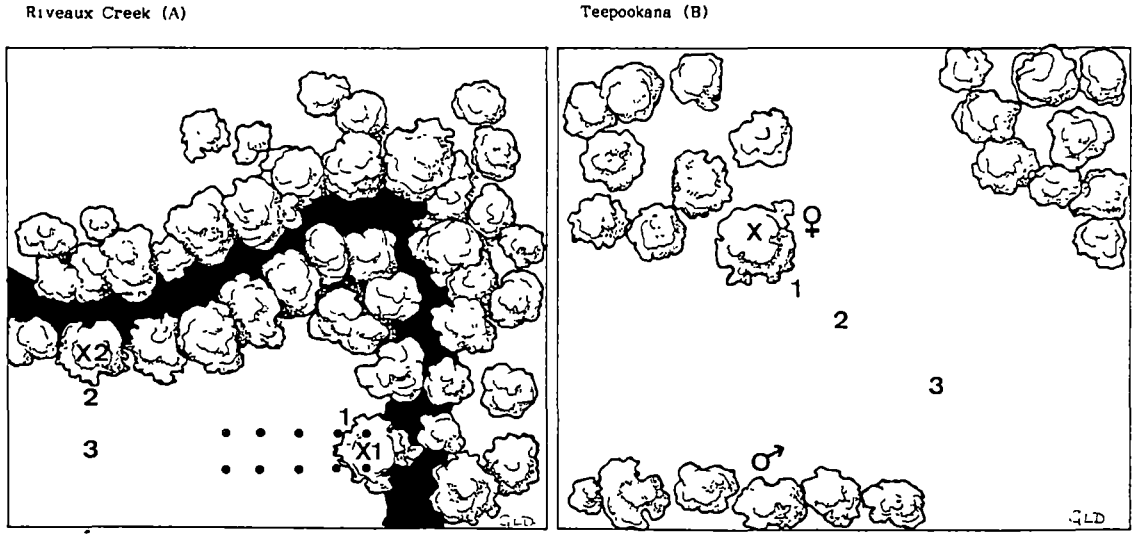
Colonisation Down Condominium Creek

Condominium Creek (Long. 146°23', Lat. 42°57') flows southwest from Mt. Anne. The creek was surveyed upstream from its intersection with the Scotts Peak Road until the first Huon pines (in this case seedlings) were found. The whole stand was surveyed and sampled. All Huon pine plants and their location in relation to the creek were recorded and the following measurements taken; height, diameter and sex (if reproductively active). Any trees located further up the creek were not investigated. Nearly all of this Huon pine population occurs on the northern side of the creek, with distribution on the southern side restricted to the upper older end of the stand. Button grass (*Gymnoschoenus sphaerocephalus*) and *Leptospermum*-dominated heath dominates the southern bank in most places.

RESULTS

Seedfall and Dispersal

Figure 1.1 The spatial relationship between Huon pine seed traps, seed trees and Huon pine dominated forest at Riveaux Creek (A) and Teepookana (B) study sites. The position of the seed traps are represented by (1), (2), (3) while the seed trees are reported by (X), (X1), (X2). The site of quadrats for soil/litter samples are also shown (•).



The trees at Riveaux Creek shed approximately 50000 seeds/m^2 whereas at Teepookana seedfall was only 2200 seeds/m^2 (Table 1.1). The seeds were collected for a longer period at Riveaux Creek and the trees had larger crowns which may

account for some of the discrepancy between sites. Both sites show a pattern of limited lateral seed dispersal. No seeds were found at a distance of one tree height at Teepookana and only eight were found at this distance over the six months of collection at Riveaux Creek.

A second season of both male and female cone production was observed at many sites. However at Riveaux Creek, female cone development was out of synchrony with male cone maturation, with the result that no seed was set.

The quantity and timing of seedfall in traps (1) and (2) at Riveaux Creek are shown in Figure 1.2. Seedfall started in February, the peak period of seedfall occurred about eight weeks later, after which seed continued to fall at a reduced rate. The last seed counts were made six months from the onset of seedfall, although the last of the seed was still dropping in December. The timing and amount of seedfall was similar for the two trees at Riveaux Creek. The two transects designed to examine distribution of the soil stored seed showed similar patterns of seed dispersal similar to those observed from the seed traps. Although more seed was collected from transect (1), little seed was found 12m from the canopy edge (Figure 1.3). The seed contained in the soil samples from under the canopy was approximately 20% of the amount recorded in the seed traps, on a per unit area basis. This suggests that substantial seed loss occurred over the eight months.

Table 1.1. The total number of Huon pine seeds collected in seed traps at the Riveaux Creek and Teepookana study sites from February to November 1989.

Site	Seed Trap Number (distance from seed tree)	Seeds/m^2
Riveaux Creek	1 (0 m)	$5.2.x\ 10^4$
	2 (0 m)	4.8×10^4
	3 (25 & 15 m)	8×10^0
Teepookana	1 (0 m)	2.2×10^3
	2 (20 m)	0
	3 (60 m)	0

Figure 1.2 Cumulative Huon pine seedfall in two 1 m^2 seed traps over time at Riveaux Creek

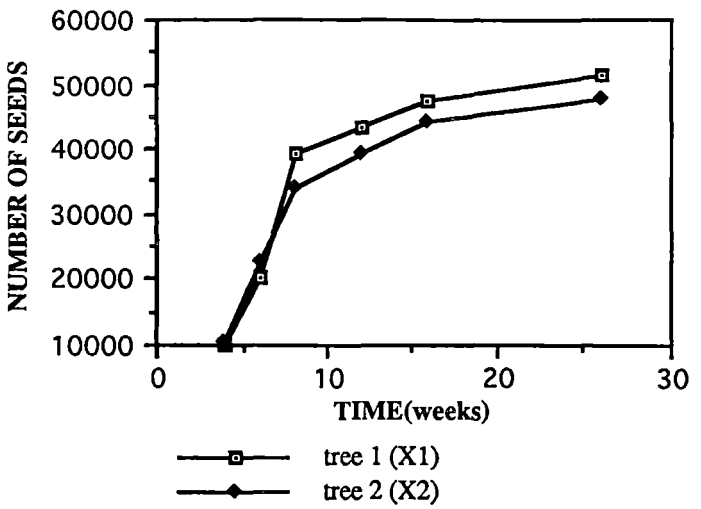
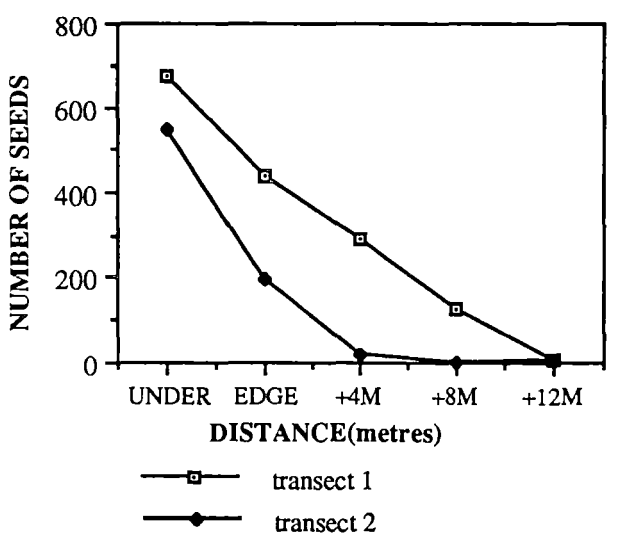


Figure 1.3 Number of Huon pine seeds in 576 cm^2 soil/litter samples along two parallel transects which extended out from under the canopy of Huon pine seed source tree (1) (see Figure 1.1a). (Edge; edge of seed tree canopy (approximately 4m from first measurement); Under; under the middle of the seed tree canopy and at the start of the transect).



Green Rosellas

Green rosellas (*Platycercus caledonicus*) were first observed feeding on Huon pine at Riveaux Creek in April 1989. There was no evidence of their presence prior to this. Such evidence consists of broken terminal branchlets found beneath trees (Brown 1984). Sightings and evidence of their presence continued until December 1989. The commencement of feeding corresponded with peak seedfall and the end of the warmer weather. All branchlets found on the ground before October bore mature female cones. This, together with direct observations, suggests that they were feeding on the seeds. From October 1989 (coinciding with maturation of young male cones and the end of the 1988/89 seed crop), green rosella activity seemed to move to the male trees where branchlets bearing mature male cones were found beneath the trees. Scattered evidence of feeding by green rosellas was observed at several sites from seedfall onwards during field work. These observations support the suggestion by Gibson (1986) that green rosellas feed on Huon pine. They appear to eat mature seeds and pollen. It is unknown what the effect of their gut is on the seeds. Green rosellas are known to carry food up to 100 m to feed (P. Brown pers. comm.) and this could offer some lateral dispersal possibilities for Huon pine.

Seed Floatation

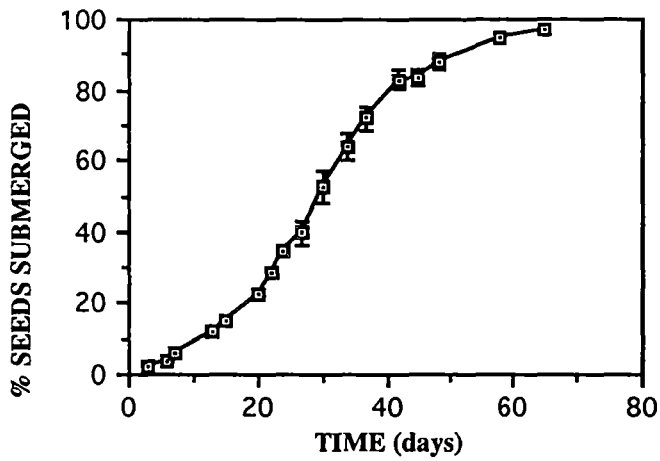
Approximately 50% of seed floated for one month and 5% stayed afloat for at least two months (Figure 1.4). If seed can stay afloat for such long periods of time, the potential distances for dispersal by water are enormous, provided viability is retained.

Squash tests for embryo deterioration indicated that seeds were usually still healthy after two months soaking (Table 1.2) regardless of whether they remained floating or were submerged. Dry stored seed from the same batch had a very high percentage of healthy embryos. Germination tests on submerged seed indicate that soaked seeds start germination marginally faster than dry stored seed.

Table 1.2. The percentage of viable embryos (determined by squash tests) in Huon pine seed used for the seed floatation experiment. Floating, submerged and air-dry stored seeds were compared

	Number of seeds tested	% Viable seeds
Floating seeds	18	78
Submerged seeds	20	80
Air-Dry stored seeds	100	99

Figure 1.4 Percentage of seeds no longer floating over time. The percentages are mean values of 3 replicates of 1000 seeds. the standard error bars are indicated.

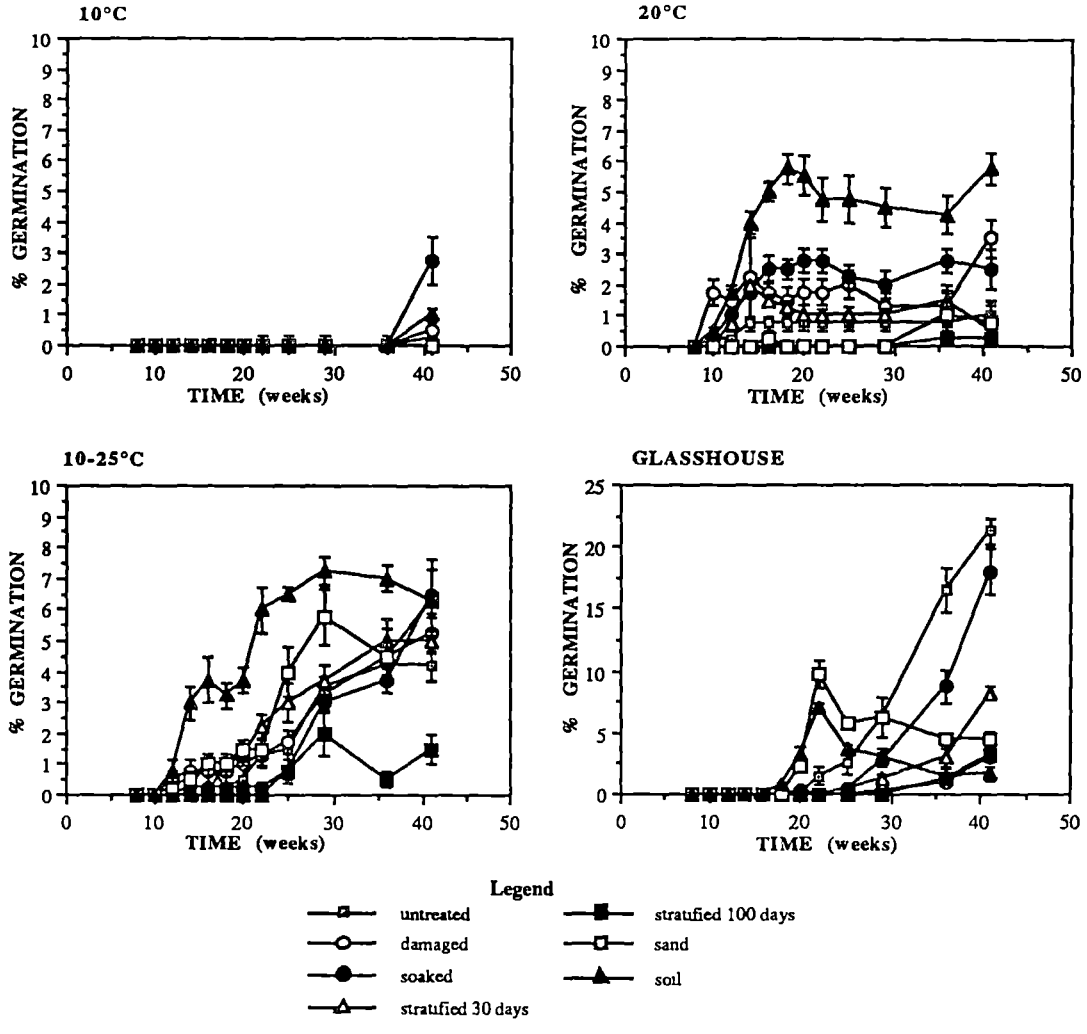


Germination

In general, germination in glasshouse trials was low with less than 20% overall germination in most treatments and less than 10% of germinants progressing to the second (root) or third (expanded cotyledon) stages. The highest germination rates were achieved in the glasshouse after week 41, by untreated seeds (25.75% total germination). The earliest germination started at week 10 at all temperatures except for 10°C. Very little germination occurred in the 10°C treatments and it only occurred after week 36 (Figure 1.5). The earliest seedlings (stages 2 and 3) appeared in week 10, from the 20°C and damaged treatment combination. At 20°C and 10-25°C the 'soil' treatment was the most successful (Figure 1.5). Initially the 'soil' and 'sand' treatments performed well in the glasshouse, but towards the end of the experiment (weeks 36-41) the 'untreated' and 'soaked' treatments surpassed them. Stratification did not improve germination.

Field records showed that germination in the field began between weeks 29 and 36 of the experiment and corresponded to the rapid increase in germination observed in some glasshouse treatments. In general the time before germination began was less in the germination experiments than in the field situation. The effects of physical treatments on germination were not constant between the temperature treatments.

Figure 1.5 Mean percentage germination of Huon pine seeds over time (weeks) under seven physical treatments, and given over four temperature regimes.



Seedling Establishment

Approximately 23% of seedlings in the field were found on logs or living trunks. Logs thus make up a sizeable minority of establishment sites (Table 1.3). Seedlings (cotyledon stage) germinated around February in 2 consecutive years at Riveaux Creek, approximately 12 and 24 months after the initial onset of seedfall. There was little seed fall in the following season so the second germination 'wave' of seedlings were from the same seed set. Compared to the amount of seedfall, germination success was very low. The timing of germination in the field coincided with a germination flush in the glasshouse treatment (under natural light conditions). Thus germination in the field appears to be coinciding with a particular daylength.

Figure 1.6 The illustration represents a transect of the Huon pine population up Condominium Creek from 0 metres to 420 metres. 0 metres represents the first occurrence of the Huon pine upstream of its intersection with the Scotts Peak Road and the stand came to a natural end at 420 metres. The occurrence of the first reproductively active male ♂ and female ♀ trees up the transect are indicated. Only Huon pine plants are represented and except in the tallest denser section all plants recorded are illustrated.

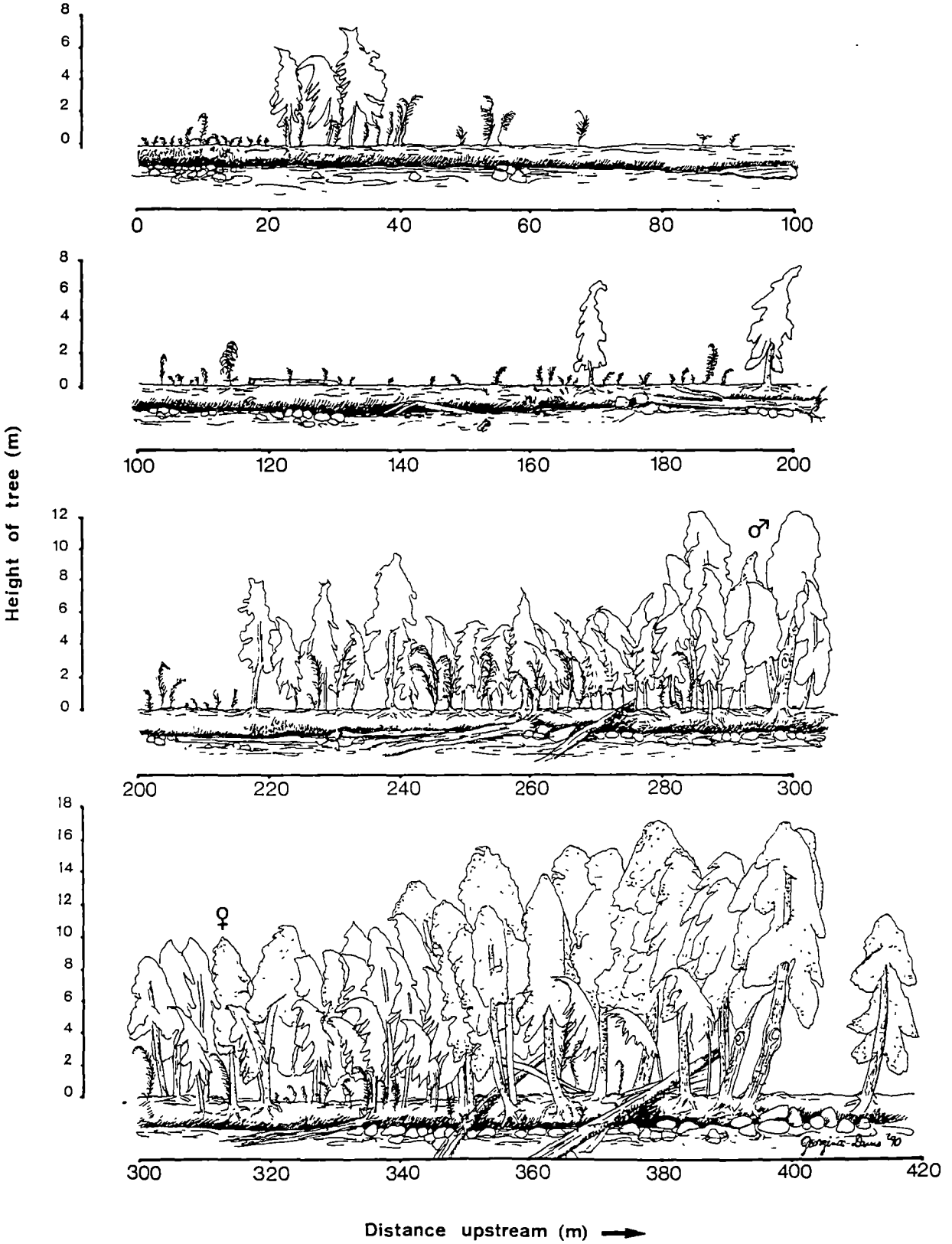


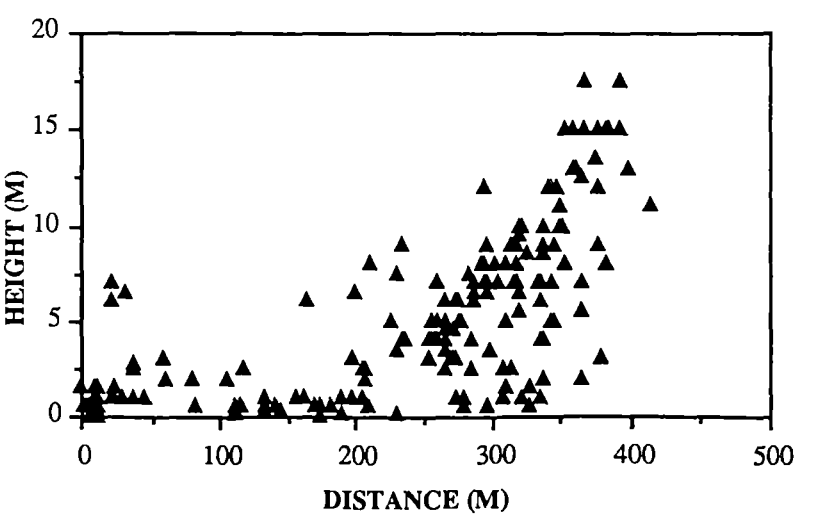
Table1.3.The number of Huon pine seedlings found on different substrate types.

SITE	No on Soil	No on Logs	Total No	Area sampled m^2
NEWALL CK	9	12	21	4560
STANLEY RIV	14	65	79	2100
GREYSTONE BLUFF	20	29	49	1200
PINE CK	0	13	13	4800
RIVEAUX CK	185	2	187	1220
DENISON RIV	10	4	14	3060
GORDON MOUTH	40	0	40	200
TEEPOOKANA	1	5	6	3936
FRENCHMANS CAP	22	0	22	216
PICTON RIV	88	0	88	1150
PIEMAN RIV	20	4	24	6682
GILBERT LEITCH	5	12	17	1196
ANNE RIV	8	0	8	880
TAHUNE RES	71	41	112	1092
CONDOMINIUM CK	71	1	72	6210
HUON RIV	138	4	142	695
KING RIV	12	0	12	975
JUNCTION CK	25	23	58	2565
EAGLE CK(Gordon)	85	27	112	2042
TOTAL	824	242	1066	44779
TOTAL % of seedlings	77.30%	22.70%		

Colonisation Down Condominium Creek

The pictogram (Figure 1.6) illustrates the relative size and abundance of Huon pine at Condominium Creek. Small seedlings and suckers were found, mostly along the bank, from the first occurrence. However, there is a distinct size class gradation from taller trees upstream to smaller ones further down (Figures 1.6 and 1.7). If conservative age estimates are made from diameter measurements (Gibson 1986) it can be estimated that colonisation 400m downstream has taken at least 200 years.

Figure 1.7 The height of Huon pine plants is plotted against the distance along the transect, which progressed upstream, at Condominium Creek. Each triangle represents a Huon pine plant. Distances as in Figure 1.6.



DISCUSSION

Seedfall

The results indicate that Huon pines can produce very large numbers of seeds in mast years. The 5 x 10^4 seeds per m^2 compares with some of the higher seedfall densities reported in the literature for other tree species (Table 1.4). The seedfall is greater than that recorded for other Tasmanian rainforest tree species.

Table 1.4. A summary of seedfall data (collected from the literature) from a variety of high seed yielding tree species. All data have been converted to the number of seeds/m^2. The data for Huon pine is from the present study.

Species	Seed number/m^2	Author
+**Lagarostrobos franklinii*	5.0 x 10^4	Shapcott A
+*Macrozamia communis*	3.5 x 10^3	Ballardie R.T. & Whelan R.S. 1986
+*Dendrocalamus strictus*	1.3 x 10^5	Janzen D.H. 1976
**Nothofagus cunninghamii*	1.3 x 10^4	Howard T.M 1973
**Eucryphia lucida*	8 x 10^2	Hickey J. *et al.* 1982
**Atherosperma moschatum*	1.2 x 10^3	Hickey J. *et al.* 1982
**Eucalyptus delegatensis*	4 x 10^3	O'Dowd D.J. & Gill M. 1984
+**Athrotaxis selaginoides*	7.7 x 10^3	Berrigan S. 1986
+*Dacrydium cupressinum*	1.2 x 10^3	Ogden J. 1985
Picea sitchensis/ Tsuga heterophylla	3.5 x 10^3	Harman M.E. & Franklin J.F. 1989

\+ mast seed year * Tasmanian species

Mast seeding is characteristic of the Podocarpaceae as well as many other tree species (Norton *et al.* 1988). Many authors (Janzen 1976) suggest that climatic conditions may trigger a mast year. In New Zealand hot dry summers are thought to be a climatic cue (Silverton 1980, Ogden 1985). Ogden (1985) suggests that periodic massive seeding may be a strategy for keeping safe sites fully stocked with long lived seedlings and may also provide enhanced opportunities for long distance dispersal and colonisation of sites which are spatially and temporally restricted. An alternative hypothesis suggests that synchronous seeding acts to satiate seed predators with an over-abundance of seed and in addition, prevents predator population growth in intervening years (Janzen 1976, Silverton 1980, Wardle 1984, Louda 1989). If predator satiation is a factor, trees out of synchrony will be strongly selected against due to a build up in predator numbers during the mast year (Janzen 1976, Silvertown 1980). Little is known of seed predators of Huon pine, although the green rosella observations are consistent with predator satiation. Since the observed

feeding, apparently large quantities of seed were left (Table 2.1). Large numbers of birds did not gather and feed on Huon pine at Riveaux Creek and there was limited evidence of green rosella activity in other stands. However, at Riveaux Creek the Huon pine seeds and pollen seemed to provide food over winter when seed from other species may have been less abundant.

The climatic conditions previous to the 1988/89 masting year seem to fit the hot, dry summer climatic cue observed in New Zealand. Masting in that season was also observed in two Tasmanian *Athrotaxis* species. The development of a second mast year in Huon pine during 1989/90, though it appears to have been less uniform, is interesting. Consecutive mast years have also been recorded in New Zealand podocarps (Ogden 1985, Norton *et al.* 1988). Good fruiting was recorded for the other Tasmanian podocarps in the 1989/90 season in Tasmania (P.B. Tomlinson pers. comm.).

The seed matured and was shed over an extended period of time. It has been suggested that an extended period of seed/fruit maturation may be advantageous for animal (e.g. bird) seed dispersal (Janzen 1983). As Janzen (1983) points out, although much seed is likely to be destroyed in the gut, some may pass through intact and depending on the habits and nature of the animal, may be dispersed to favourable germination sites. Green rosellas may act as both predators and dispersers of Huon pine seed. The pattern of Huon pine seedfall may minimise predator effects by masting and maximise dispersal by extending the seed maturation and shedding period.

Dispersal

Lateral dispersal by wind is very limited as evidenced by the seed trap and soil/litter data. Dispersal by this method is similar to that observed in other podocarps (Norton *et al.* 1988) but less than that recorded by Hickey *et al.* (1982) for other Tasmanian rainforest tree species. The data support the view that the limited lateral dispersal of Huon pine increases the time taken for colonisation of surrounding vegetation (Millington *et al.* 1979, Davies 1983, Gibson 1986). Given that 'BIOCLIM' data suggests Huon pine could at present survive in a broader habitat range than it does, (Gibson 1986) poor lateral dispersal may be an important limiting factor. However, the Condominium Creek example demonstrates that downstream colonisation is slow even where dispersal is good, and many areas of superficially suitable habitat may not be colonised. Davies (1983) noted the patchy Huon pine occurrence on the Denison River and suggested it may coincide with limestone rock types. However,

rock type does not explain the conditions at Condominium Creek. Fire may partly explain the pattern observed at this site, but other factors such as seedling establishment require further examination.

The amount of seed retrieved from soil samples gave an indication of the size of the soil seed bank and suggested that most seed was lost from the soil within a few months of falling. Baker (1989) found that seed of some species from moist habitats survives better if kept moist. It is possible that Huon pine seeds found in moist locations could remain viable and germinate over an extended period of time. Thus, in non-mast years, seeds from the previous season may germinate when favourable conditions occur. There is some evidence that this may occur, since seed germinated two years after shedding at Riveaux Creek, and small numbers of cotyledon-size seedlings were found at several sites during the study. These latter seedlings must have resulted either from limited non-mast year seedfall, remained at cotyledon stage for several years or germinated after the major seedfall and first subsequent germination wave.

Germination and Establishment

The germination experiments demonstrated that germination can occur over an extended period of time. The low germination success may be due to poor seed viability or seed dormancy. Germination in the glasshouse experiment began considerably earlier than in the field, probably due to the warmer growth conditions. However, there was a flush of germination in the 'Glasshouse' treatment (natural day length) coinciding with germination in the field. Since germination in the field at Riveaux Creek occured in the same limited period in two consecutive years, a stimulus related to day length may trigger dormancy release in this case. The success of the 'soil' treatment suggests that elements present in this medium may enhance germination. The success of the 'soaked' treatment indicates the role water may play in enhancing germination. The germination success of seeds soaked for two months during the floatation experiment supports this view and may suggest that seeds which are water dispersed have a greater chance of successful germination than seeds laterally dispersed by wind.

The floatation capabilities of Huon pine seeds (Figure 1.4) appear far greater than those reported for other podocarps (Ogden 1985), the most buoyant being the small seeded species which are reported to be "buoyant until the waxy coat becomes wetted by turbulence" (Preest 1963). The long floatation period, coupled with the large number of seed falling directly into water, makes the potential water dispersal

of Huon pine seeds enormous. This form of dispersal is limited to the floodline or to the edge of water courses. The Condominium Creek example shows that even with good dispersal, expansion of this stand appears to have taken at least 200 and possibly 400 years or longer based on stem diameter measurements and average diameter increments (Gibson 1986).

Many of the seedlings found on soil (Table 1.3) were observed to be growing on mossy mounds, which may be the more stable sections of the bank and consequently less prone to erosion due to flooding. The data on seedling substrates demonstrates that logs are not major germination sites, as they are reported to be in other habitats and for other species (Read and Hill 1983, Harman and Franklin 1989). Harman and Franklin (1989) suggest that even where logs do form the major germination sites, these sites are not necessarily suitable for establishment of mature trees, due to competition between trees and the inadequacy of the substrate for larger root penetration. This contrasts with Read and Hill (1983) who suggest that in regenerating forests logs may provide competition free sites.

Playford and Dettman (1979) have suggested from pollen data that Huon pine may have been much more widespread in the past, and, together with other authors (MacPhail 1979, Colhoun and Van de Geer 1987), have suggested that Huon pine survived the last glaciation in isolated refugia, with the present distribution resulting from an expansion from these refugia. Macrofossil evidence does not support the view of a past widespread Huon pine distribution (Hill and MacPhail 1985, Wells and Hill 1989). Hill (1990) suggests that fossil pollen from the Tertiary period may belong to a suite of Podocarpaceae species which pre-dated Huon pine. Even if the past climate favoured an expanded distribution, range extensions, particularly uphill and away from rivers, would have been very slow. The origin of isolated high altitude sites such as at Lake Vera, Mt. Read and Greystone Bluff have not, in my view, been satisfactorily explained, although green rosellas or their predecessors may have played an important role.

Conservation and Management

Since most effective dispersal appears to be down water channels, stands at the headwaters of catchments may represent stocks from which downstream regeneration may be possible. Thus they represent key stands to be conserved. In addition, the stands on river flats and away from major stream flow should be targeted for conservation, as they are not likely to regenerate easily from surrounding stands since lateral dispersal is extremely limited. Some of these stands may have

arisen from past river courses or flooding patterns. Since many rivers are now controlled by upstream dams, flooding is a less likely avenue of seed dispersal. Riverbank integrity is important for the germination and establishment of seedlings, as most seedlings have been found on soil and sandy/mossy banks and most trees are found along river edges. Activities which degrade river banks will reduce the survival of Huon pine. For example, continual deposition of material can smother young plants, and wave action which erodes the bank can undermine root systems and wash away seedlings and small trees. Vegetated buffer zones around Huon pine stands, particularly along water courses, would also buffer against fire and allow establishment of Huon pine seedlings by maintaining a moist environment.

CHAPTER 2 :

Reproductive, sex and size structure of Huon pine stands.

INTRODUCTION

Stand structure is often used to help deduce regeneration patterns and requirements for particular species (e.g. Cullen 1987, Gibson and Brown 1991, Stewart 1989, Veblen 1989). Regeneration depends on the potential stocking density of sites, which may be limited by physical or environmental constraints (Antonovics and Levin 1980). It is also affected by species' longevity, mortality and hence turnover rate. It will be different depending on whether the population is maintaining itself *in situ* with time, or moving in response to a changing environment. Ultimately however, regeneration depends on reproductive expression and the fitness and survival of offspring (Lechowicz and Blais 1988). The relative proportions of sexually or vegetatively produced trees may affect the level of inbreeding or outbreeding within a population. The interactions between reproductive expression, gender, age, genetic compositions, and the turnover of trees within populations will ultimately affect the ability of a species to respond to changing conditions.

Many studies have related reproductive expression to structural factors such as light availability, density, and age or size structure (Ross and Pharis 1987, Sakai and Oden 1983, Sedgely and Griffin 1989, Putwain and Harper 1972). Structural effects may also influence the ratio of males to females (Lloyd and Bawa 1984, Freeman *et al.* 1976). For example in some species there is differential developmental expression of gender, so that the male to female balance can be affected by the age structure of populations (Ross and Pharis 1987, Lloyd and Webb 1977). The density of stands may also act to favour one sex over another (Lloyd and Bawa 1984, Putwain and Harper 1972, Freeman *et al.* 1976). In some plants there are differential energy costs for reproduction in each gender type, since for example continued energy is required for the development of seeds (Putwain and Harper 1972, Lloyd and Webb 1977). Environmental stress has generally been found to favour males, and female dominated populations have been found on more mesic or fertile sites (Freeman *et al.* 1976, Lloyd and Webb 1977). There may be differential energy costs associated with sexual reproduction verses vegetative reproduction (Putwain and Harper 1971, Lechowicz and Blais 1988, Koella 1988, Ross and Pharis 1987). Sex ratio can also be altered by the unequal success of vegetative clones of one or other gender

(Putwain and Harper 1972, Lloyd and Bawa 1984, Melampy and Howe 1977). Although the genetic proportions of males and females in a population may be equal, differences in their reproductive expression may lead to unbalanced sex ratios which can be inconsistent between seasons (Ross and Pharis 1987, Freeman *et al.* 1976, Primack and Macall 1986). Initiation of reproductive activity may respond to different environmental stimuli in each sex (Melampy and Howe 1977, Ross and Pharis 1987). Indeed, environmental factors may override genetic sex determination, causing sex change or expression of both sex types (Freeman *et al.* 1976, Ross and Pharis 1987). All these factors have implications for both reproductive and evolutionary success.

Huon pine is a dioecious species and therefore an obligate outcrosser. However its propensity for vegetative reproduction has been noted by many workers (Davies 1983, Gibson 1986, Pedley *et al.* 1980). While dioecy is widespread but infrequent in angiosperms, being found more often in tropical floras (Bawa 1980), it is fairly evenly distributed amongst gymnosperm families. The distribution of dioecy and monoecy in gymnosperms corresponds roughly to seed dispersal mechanisms (Givnish 1980). The family Podocarpaceae, of which Huon pine is a member, is unusual in having approximately equal numbers of monoecious and dioecious genera (Givnish 1980). Dioecy has often been proposed as a mechanism to ensure outcrossing and hence maintain genetic diversity (Bawa 1980), but this may be a product rather than cause of its evolution (Bremmerman 1985).

Most studies of sex expression have been undertaken on short-lived, fast growing species which often grow in highly disturbed environments (Putwain and Harper 1972, Lloyd 1980). The investigation of long-lived slow growing plants are important if the relevance of other studies is to be evaluated. Studies on reproductive biology of plants are often undertaken using only a few sites. When a species, such as Huon pine, inhabits a variety of environments, variable responses can be expected. By studying the breadth of responses we have a greater pool of information from which to assess the nature of the patterns or the limitations in the reproductive behaviour of a particular species. This better equips us to predict the kinds of responses a species may have to changing future conditions.

Spatial patterning of reproductive expression and sex will affect the randomness or otherwise of gene flow within populations (Sakai and Oden 1983). Spatial pattern may have an influence on sex expression itself (Sakai and Oden 1983). The spatial patterning between and within sites of both reproductive activity and sex expression, may shed light on the factors determining reproductive and sex expression in Huon

pine, if considered in conjunction with, structural, ecological and environmental variation.

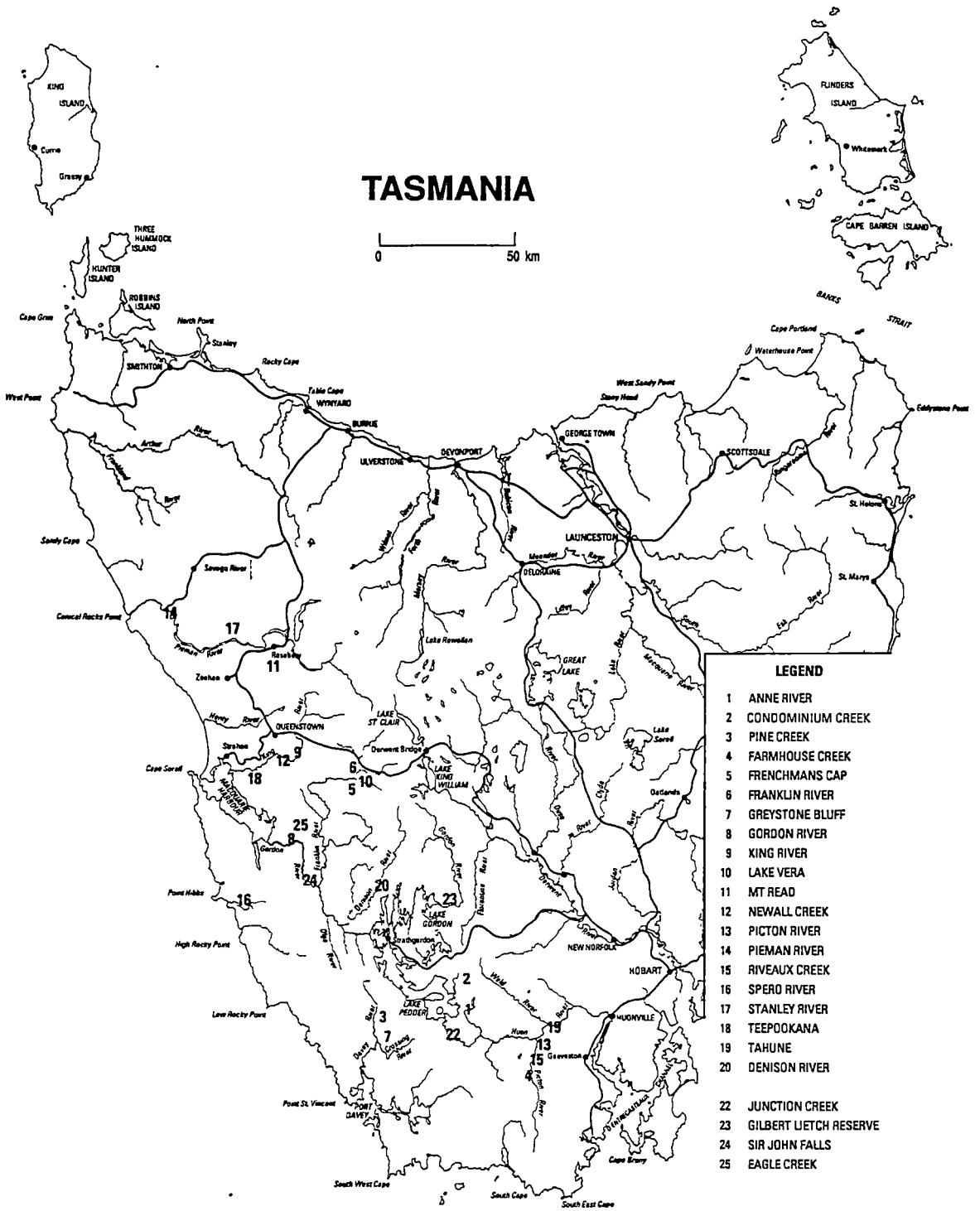
Although the dynamics of stand structure and reproductive expression and success are intimately entwined, seldom are they studied together. This chapter investigates the amount and variability of reproductive and sex expression, at sites covering the present ecological and geographical range of Huon pine. The relationships between ecological or environmental conditions, and structure, reproduction or sex expression are also examined along with the spatial relationships (if any), between reproductive male and female trees and non-reproductive trees within stands.

FIELD METHODS

A survey of Huon pine populations was undertaken across their ecological and geographic range (Figure 2.1). Part of the survey sampled twenty seven locations (Figure 2.1) for reproductive activity, sex expression and stand structure. Two locations consisted of several nearby sites. Seven sites were sampled at the Pieman River and three at the mouth of the Gordon River. Huon pine trees were sampled along belt transects. Within the transect area all Huon pine plants were recorded and allocated a unique number. Their locations were noted as co-ordinates (length and breadth) relative to the transect line. The height and diameter of each Huon pine plant were recorded, and trees were assessed for the presence and gender of cones. In cases of multi-stemmed trees where it was clear that all shoots arose from the same base the dominant stem was used for all measurements, but other major stems were also noted. If trees were unconnected to ground level they were treated as separate stems, although possible connections between trees were noted where they were growing in clumps. Species lists of vascular plants were compiled for twenty-three of the locations including three of the Pieman River sites.

Transects varied in size depending on the size, shape, and density of the stand, and on the time taken for sampling, since at some sites time was restricted. Transects varied in length by multiples of the minimum length of thirty metres. Their width varied from five to forty metres. Where possible whole natural groupings (stands) of Huon pine trees were encompassed. Tree co-ordinates were not recorded at some sites. For one section of the King River transect and at the Gordon mouth sites, only height, diameter, reproductive status and sex were recorded. At the Franklin, Birches Inlet and Gordon River sites, only the reproductive status and sex of trees were recorded and at Lake Vera an additional transect was undertaken on which these were the only measurements.

Figure 2.1 The locations of Huon pine stand structure study sites.



DATA ANALYSIS

Diameter and Height

All Huon pine plants at each site were classified into diameter and height classes (Figure 2.2 and Appendix 2). The numbers were converted to percentages of the population in each class to standardise the different sample sizes between sites. The height classes covered three metre intervals (Appendix 2). However it was not appropriate to make all diameter classes of equal size due to the extended range and distribution of diameters occurring in this species. Diameter class boundary ranges of 2, 5, 10 and 20 centimetres were used. The classification system used does not have even classes, and thus pulses in diameter distributions need to be interpreted with care. Diameter size class distribution histograms were assessed visually. The density of Huon pine trees in each site was calculated from transect data.

Climatic Variables

The BIOCLIM program (Busby 1991) was used to generate sixteen synthetic climate profiles for each site from their geographic co-ordinates and altitudes. These synthetic climatic profiles were then included as site variables in a Principal Components Analysis (PCA) using SAS/STAT® procedure FACTOR (SAS Inst.Inc. 1990). The first four principal components produced by the PCA analysis together account for 99.4% of the variation in the standardised data. These were treated as composite bioclimatic variables in further analyses.

Reproduction

A Chi square test was undertaken on the pooled data from all locations to test whether the ratio of males to females differed from the expected ratio of 1:1. Chi square tests were also undertaken for each location to test for deviances from this ratio. Plants for which gender could not be determined were excluded from the analyses. An analysis of deviance was also undertaken (GENSTAT V 1987) on all sites to see if any individual sites differed significantly from the expected ratio of 1:1, and to determine in which direction the deviation occurred. This method is more sensitive than individual Chi square tests on sites and takes into account random variation in this ratio in a group of sites. Bisexual (monoecious) trees were observed in some locations. The overall proportion of reproductively active trees which were monoecious was calculated from data pooled from all sites. This figure was used to

calculate the expected frequency of bisexual trees at each site and Chi square tests were performed to identify those locations with significantly greater proportions of bisexual trees than expected. The level of reproductive activity was estimated as the proportion of trees with cones present. Only Huon pine plants 1 metre tall or higher were included in analyses, since cones were not recorded on any Huon pine plant less than 1 metre tall. Smaller trees were therefore not considered to contribute to the reproductive population. The total percentage of reproductively active trees was calculated from pooled data and this was used as the expected proportion of reproductively active trees in any individual site. Chi square tests were undertaken on each site to determine any significant deviation from this proportion of reproductively active trees.

Relationships Between Site Variables

The first three of the PCA generated composite bioclimatic variables, geographic locations, stand density measures and reproductive variables (Table 2.1) were analysed for possible correlations using SAS/STAT® procedure CORR using Spearmans rank correlation (SAS Inst. Inc. 1990). The use of Spearman's rank correlation coefficient is appropriate where distributions are markedly skewed (Conover 1980).

Table 2.1 Variables used in Spearmans Rank correlation matrix

Variable	Description
Easting	Universal grid reference Easting (100 metre units)
Northing	Universal grid reference Northing (100 metre units)
Altitude	Altitude (metres)
Climate1	Climatic principal component 1 generated from PCA analysis of BIOCLIM generated synthetic climate profiles.
Climate2	Climatic principal component 2 " "
Climate3	Climatic principal component 3 " "
Density	Density, the number of Huon pine trees per square metre.
Seedling Density	Seedling density, the average number of Huon pine seedlings per square metre. (all Huon pine plants less than one metre tall were classed as seedlings)
Prop Females	The proportion of dioecious reproductively active Huon pine trees which were female.
Reproductive	The proportion of reproductively active Huon pine trees.

Dissimilarity matrices were generated for several variable types. Distance matrices were generated from, geographic grid co-ordinates, the proportion of female trees (see Table 2.1) and the proportion of reproductively active trees. Huon pine density and Huon pine seedling density were also used to generate a dissimilarity matrix (Table 2.1). Sites were categorised into classes for each of eight variables; disturbance, logging, canopy structure, surrounding vegetation, position of the stand,

canopy height, age structure, type of stand (Table 2.2). These classes were then standardised to scores from 0 to 1 for use in the generation of composite dissimilarity matrices. Five of these variables related to the disturbance or the amount of light penetration in stands, and were used together to generate a composite disturbance dissimilarity matrix (Table 2.2). The other three variables, (canopy height, age structure and type of stand), related to stand structure and were used to generate a composite structure dissimilarity matrix. The four BIOCLIM/PCA generated composite climatic factors were used to generate a climatic distance matrix. Squared Euclidean distance was used to compute all of these distance matrices using the PATN statistical package (Belbin 1991). The DECODA statistical package was used to calculate a dissimilarity matrix based on vascular plant species presence or absence using the Bray-Curtis distance measure (Minchin 1990). These dissimilarity matrices were paired in all possible combinations and analysed using the Mantels' test (1967) which was undertaken on a program based on that published by Manley (1985).

The Mantels test is a test for association between pairs of distance matrices (Manley 1985). There is no assumption of normality of the data and different types of distance measures can be used (Manley 1986). However only linear relationships are detected (Heywood 1991). The significance of correlations are tested by comparison of G, an approximately standard normal deviate of the test statistic Z, with a randomly generated distribution of G values which approximates a normal distribution (Heywood 1991, Manley 1985).

Table 2.2 The variables used to generate distance matrices for disturbance and structure, and the categories into which each variable was scored.

Disturbance	
Surrounding vegetation type	1 = closed forest, 2 = open forest
Canopy type	1 = continuous, 2 = patchy, 3 = emergents
Disturbance	1 = undisturbed, 2 = some disturbance, 3 = greatly disturbed
Logging history	1 = unlogged, 2 = light logging, 3 = heavily logged
Position of the stand	1 = on major water course, 2 = on minor water course, 3 = not on water course
Structure	
Type of stand	1 = nonlinear, 2 = fringing creek with closed canopy in closed vegetation, 3 = riverine fringe with one open side, 4 = riverine fringe with closed canopy in open vegetation, 5 = emergent clumps
Diameter distribution	1 = continuous, 2 = excess first size class, 3 = reverse j, 4 = reverse j with a pulse, 5 = one pulse, 6 = two pulses
Height	Canopy height classes from 1 - 9 with a 3 m interval

Spatial Autocorrelation

Spatial autocorrelation analysis tests whether the observed value of a variable at one point or locality is independent of values of the variable at neighbouring points or localities. If dependence exists the variable is said to exhibit spatial auto correlation (Sokal and Oden 1978a). For the purposes of this type of analysis the distribution of points is considered as given (Sokal and Oden 1978a). Both nominal and interval data may be used for these analyses.

Spatial autocorrelation was used to determine whether sites with similar proportions of females and of reproductively active trees were randomly distributed (Sokal and Oden 1978a). For example, to determine whether there was random distribution of mostly female stands, or whether they tended to occur in regions together, and also to determine if there was any broadscale grouping of more or less reproductively active sites. Populations within a thirty kilometre radius were defined as neighbours for the analyses. A thirty kilometre radius distance was chosen to be large enough for sufficient numbers of populations to be neighbours, and to give statistically meaningful results, given the sampling points and Huon pine distribution, but also to be small enough to differentiate regions.

The relationships between individual pairs of trees within sites was also investigated to see if there was structuring or pattern in the distribution of reproductive and nonreproductive Huon pine trees, or between male or female trees. The actual co-ordinates of all Huon pine trees within the sample sites were used for the analysis. Sites with very low proportions of reproductively active trees were excluded from the analysis. Huon pine trees within a ten metre radius were considered to be 'neighbours' for the purposes of this analysis. This area was based on field observations, and was estimated to be the approximate area of influence of an average individual Huon pine tree. The distance also took into account the density of Huon pine trees so that there would be sufficient 'neighbours' to give statistically meaningful results. The variation in autocorrelation, and hence relationships, between trees that occurs with increasing distances between pairs of trees was analysed by assessing for autocorrelation between 'neighbours' within increasing distance classes. These had an annular increase of five metres.

In both between site and within site analyses connections between neighbours were given a binary score of 1 if 'joined' by the pair definition, or 0 if not 'joined', within each distance class. Thus for example all pairs of females or female dominated sites were assessed for the number of times they co-occured within a distance class. This

number was compared with the number of joins expected, if reproduction and sex were randomly distributed, and with the variance for each type of join (Sokal and Oden 1978a). If there was a significant excess of joins between a pair type (e.g. females), those pair types would be said to be positively autocorrelated, a deficiency of joins would indicate negative autocorrelation (Epperson and Clegg 1986). Interval data were used for the population analysis, in this case significance is assessed using the test statistics of Moran's I or MacGeary's C (Sokal and Oden 1978a). Nominal data were used for the transect spatial autocorrelation analysis. Significance is then assessed by the estimation of the standard normal deviate (SND) which is compared to a T distribution (Sokal and Oden 1978a). Corrections for small sample sizes and small degrees of freedom are given by Sokal and Oden (1978a). However generally SND = (obs-exp)/$\sqrt{}$var and the degrees of freedom are given by $2(exp)^2/\sqrt{}$var (Sokal and Oden 1978a). Both sets of data were analysed for spatial autocorrelation using a program written by M. Zalucki based on the algorithms given in Sokal and Oden (1978a).

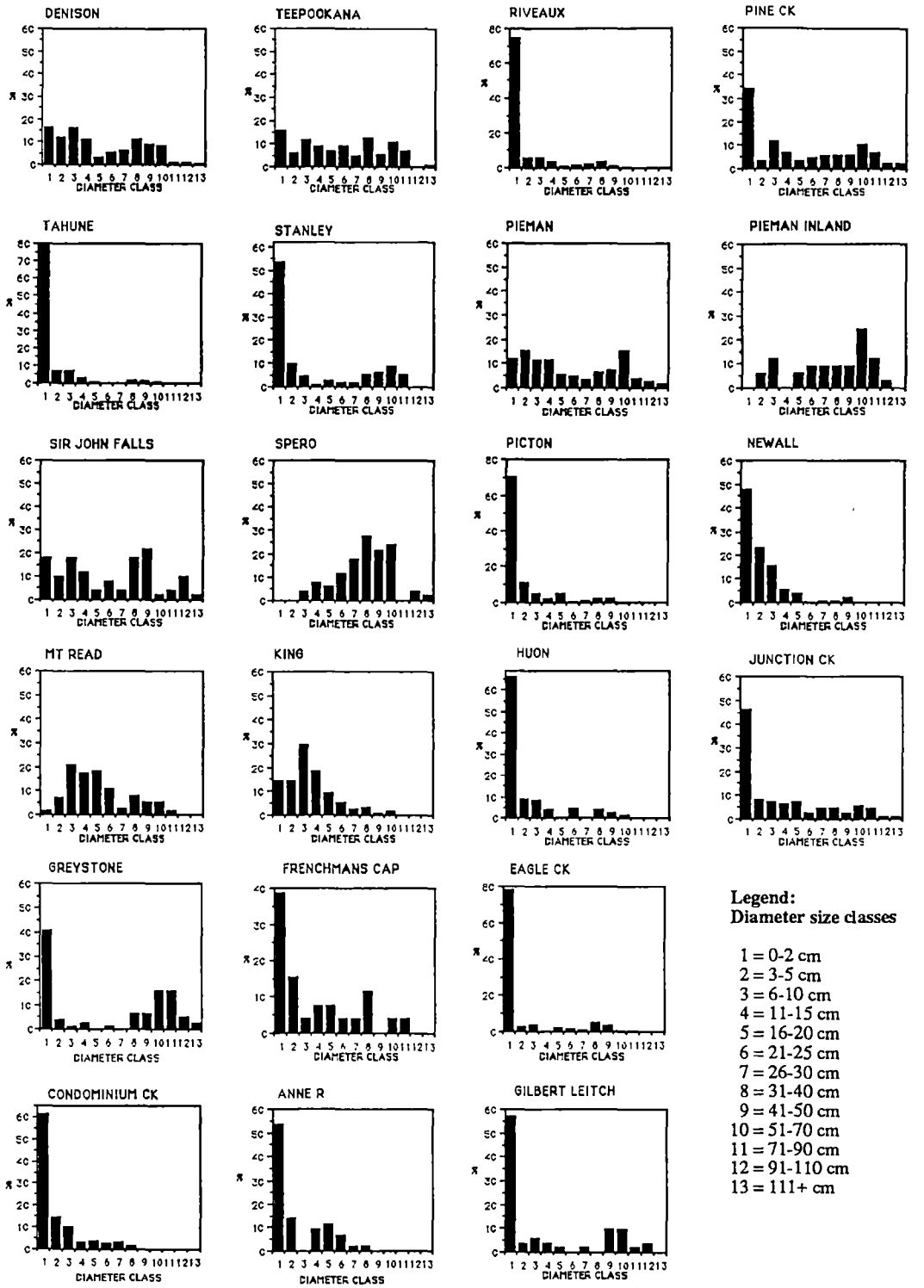
All transects were represented graphically as scatter plots mapping location, gender status and diameter. These plots were used as an aid in interpretation of spatial autocorrelation results.

RESULTS

Structure

The age and size distributions of Huon pine trees were quite variable at different sites as can be seen by diameter and height histograms (Figure 2.2 & Appendix 2). This variation suggests a range of different regeneration regimes. The structure of stands is often affected by the amount of disturbance experienced, in this study those stands with broadly similar levels of disturbance are also of similar structure (Table 2.3). Some patterns in stand structure could be determined. Some sites had large proportions of small size classes and their size distributions approximated a 'reverse J' curve typical of negative exponential growth (Hett & Loukes 1976). These sites were located on river or creek fringes where there is higher light penetration. The Frenchman's Cap stand was an exception (Figure 2.2) as the Huon pine occurs as emergent clumps amongst shorter alpine vegetation. More even size distributions were also found (e.g. Teepookana, Denison, Pieman, Sr. John Falls). Some sites had a greater proportion of older trees such as Greystone Bluff and Stanley River and those on the flats of the Spero River. Populations with even or pulsed size distribution

Figure 2.2 The diameter size class distribution at each study site. The data is plotted as percentages of all diameters in a site.



(Figure 2.2) were mostly found away from open river fringes, usually on river flats or narrow drainage channels, where there was a more closed canopy. For example the stand at Pine Creek was on a small drainage channel, with little bank structure, in a headwater basin which drained into Pine Creek itself. The Pieman River stands and the King River stand were notable exceptions. The absence of many small plants on the Pieman River (Figure 2.2) may be explained by the presence of mining tailings which form a hard crust on the banks. The structure of the King River stand is not as easily explained. However the stand was very heterogeneous and some portions were dominated by saplings of 6-10 cm in diameter, whilst others were dominated by large old Huon pine trees. This structure may represent past patchy pulses of regeneration (Figure 2.2). The structure of populations is sometimes more apparent when diameter and height histograms are both used. For example, the dominance of larger trees at Greystone Bluff becomes more apparent, as does the dominance of both seedlings and canopy trees at the Gilbert Leitch site.

Table 2.3 Summary of Mantels G values for tests comparing pairs of distance matrices between Huon pine sites. G=(Z-expected value)/standard error.
*** significant at the P< 0.05 level.**

matrices	reproduction	females	density	structure	disturb	geogr	species
females	0.48						
density	-0.66	-0.10					
structure	-1.48	-0.01	-0.37				
disturbance	*3.78	-0.36	1.28	*2.73			
geography	0.20	-0.46	*2.31	-1.49	1.66		
species	0.58	*2.25	0.81	1.25	1.26	0.46	
climate	-1.41	1.25	0.66	0.48	*3.46	0.23	*3.04

The density of Huon pine trees varied between sites from 0.014 stems per square metre at Sr. John Falls, to 0.147 stems per square metre on the Huon River site (Table 2.4). Sites with similar Huon pine density tended to be located close together, as shown by the significant positive correlation between geographic distance and density dissimilarity matrices (Table 2.3). Both Huon pine tree density and Huon pine seedling density tend to be greatest in the south eastern part of the Huon pine distribution (Table 2.5). Here populations are usually small, river fringing stands, surrounded by more open vegetation. The greater densities are due to large numbers of small trees (Figures 2.1&2.2). Stands in the north west of the Huon pine distribution, such as the Pieman River, Mt. Read, Spero and Stanley (Figure 2.1), often have fewer small trees. Therefore the lower densities of north western sites are the result of fewer larger trees dominating the stands (Figure 2.2). This geographical trend in density is also correlated with a climatic trend (Table 2.5) where tree density and seedling density are negatively correlated with the second composite climatic

Table 2.4. Density, reproductive activity and gender allocation in Huon pine study sites.

Site	Sample size No of trees	Density (No /m^2)	Female:Male ratio	%reproductive activity	% bisexual
Pieman	158	0.024	50:24 (F)	53.8 (H)	7.0 (H)
Teepookana	111	0.028	33:8 (F)	36.0 (ns)	0.0
Greystone Bluff	53	0.044	8:5 (ns)	28.3 (ns)	3.8
Pine Creek	74	0.015	15:12 (ns)	36.5 (ns)	0.0
Frenchmans Cap	27	0.125	6:2 (ns)	29.6 (ns)	0.0
Denison	196	0.064	18:15 (ns)	17.3 (L)	0.5
Newall Creek	184	0.040	4:0 (ns)	2.2 (L)	0.0
Stanley	82	0.039	3:10 (M)	17.1 (L)	1.2
Picton	105	0.091	11:16 (ns)	26.7 (ns)	0.9
Riveaux	379	0.031	45:20 (F)	18.5 (L)	0.3
Anne	43	0.049	9:6 (ns)	34.9 (ns)	0.0
Condominium	205	0.033	17:3 (F)	9.8 (L)	0.0
Spero	139	0.052	19:41 (M)	33.1 (ns)	1.6
Huon	102	0.147	5:22 (M)	27.5 (ns)	2.9
Mt. Read	101	0.118	0:67 (M)	53.5 (H)	0.0
Tahune Res.	63	0.058	13:9 (ns)	21.2 (ns)	0.0
Gilbert Leitch	33	0.027	5:2 (ns)	21.2 (ns)	0.0
Sir John Falls	79	0.014	8:14 (ns)	27.8 (ns)	0.0
Eagle Creek	79	0.039	15:9 (ns)	35.1 (ns)	2.5
Junction Ck	74	0.029	15:13 (ns)	37.8 (ns)	0.0
King River	343	0.35	67:59 (ns)	36.4 (H)	0.6
Gordon mouth	53	-	23:15 (ns)	75.5 (H)	3.8
Gordon River	102	-	51:45 (ns)	95.0 (H)	0.0
Lake Vera	102	-	17:4 (F)	20.6 (ns)	0.0
Franklin	36	-	9:1 (F)	27.8 (ns)	0.0
Farmhouse Ck	20	-	6:4 (ns)	50.0 (ns)	0.0
Totals	**2743**		**463: 411 (ns)**	**31.8**	**1.1**

Legend: Significance Levels ($P < 0.05$)
(F) significantly more females
(M) significantly more males
(H) significantly higher reproductive activity
(L) significantly lower reproductive activity
(ns) not significantly different from expected

factor which has its negative extreme in the south west (Table 2.5). A climatic change between the north west and the south east is reflected in the vegetation types present. Climatic similarity also reflects the degree of disturbance (Table 2.3), but areas prone to more storms and wind, would also be more disturbed due to windthrow and flooding.

Reproductive Activity

The overall average proportion of reproductively active trees was approximately 30% (Table 2.4). Ten of the twenty five sites varied significantly from this level of reproductive activity (Table 2.4). There were proportionately more reproductively active trees at five sites (Pieman River, King River, Mt. Read, Gordon Mouth, Gordon River). While five sites had fewer reproductively active trees in their populations (Denison, Newall Creek, Stanley River, Riveaux Creek, Condominium Creek). There were no significant correlations between reproductive activity and geographical location or proximity (Tables 2.3 &2.5).

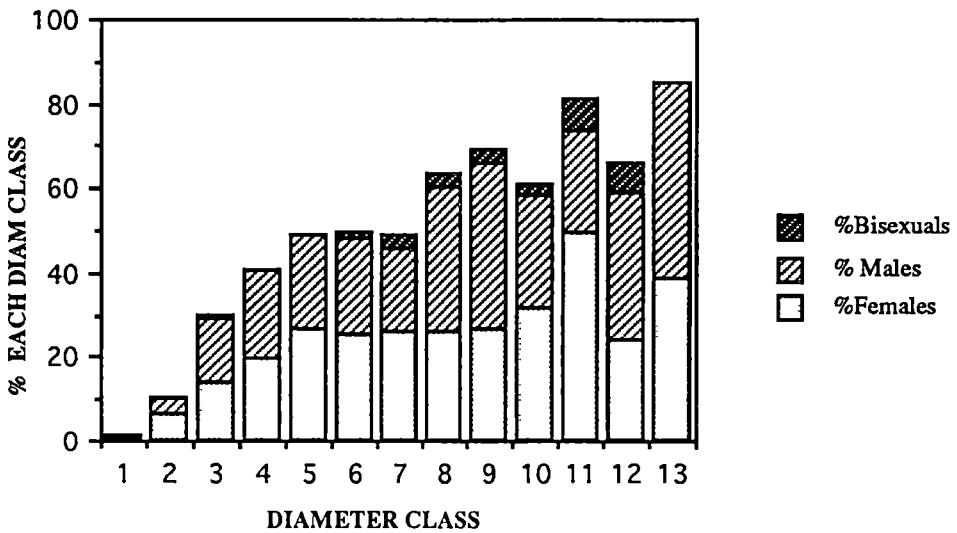
Table 2.5. Summary of correlations between reproductive, ecological and environmental variables as assessed by a Spearmans Rank Correlation analysis. The probability of each correlation significant at ($p<0.05$) is given, with (+) or (-), denoting the sign of the correlations.

Variables	North	Altitude	Climate1	Climate2	Climate3	Density	SDen	Female	Reprod
Easting	(-) 0.0001	(+) 0.0053		(-) 0.0001	(+) 0.005	(+) 0.028	(+) 0.0001		
Northing	******			(+) 0.0001	(-) 0.0041	(-) 0.0487	(-) 0.0002		
Altitude		******	(+) 0.0001	(-) 0.0051					(-) 0.0089
Climate Factor 1			******						
Climate Factor 2				******		(-) 0.0332	(-) 0.0005		
Climate Factor 3					******		(-) 0.0545		
Density						******	(+) 0.0312		
Seedling density							******		(-) 0.0345
Proportion females								*****	
Proportion Reproductive									******

The relative proportions of reproductively active trees increases with tree size (Figure 2.3). That is, the older the tree, the more likely it is to be reproductively active. This is not merely a reflection of the overall size distribution of Huon pine trees (Figure 2.4). Therefore, it is useful to compare size distributions with trends in reproductive activity. The six sites with significantly greater proportions of reproductively active trees, also had greater proportions of larger diameter trees (Table 2.4 and Figure 2.2). However, not all sites with a larger diameter bias have greater levels of reproductive activity. Four out of the five populations with significantly fewer reproductively active trees (Table 2.4), have large numbers of

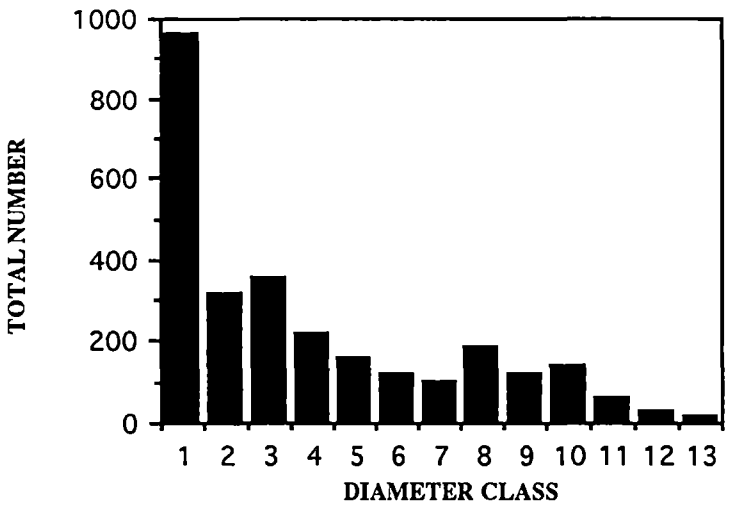
individuals in the smallest size classes (Figure 2.2). The significant negative correlation between seedling density and reproductive activity confirms this trend (Table 2.5). However, the trend was not observed in the other less reproductive site (Denison), and not all sites with large numbers of seedlings were less reproductively active.

Figure 2.3. The percentage of each gender class in each diameter class is pooled, to give the overall percentage of reproductively active trees in each Huon pine diameter class.



Reproductive activity appears to be associated with light availability. This was observed in the field in relation to the overall abundance and distribution of cones. Dense stands with small tree crowns generally appeared to be less reproductively active. This explanation accounts for all of the less reproductively active stands (Table 2.4). For example, even though part of the stand at the Denison Reserve was on the river fringe, the forest canopy was dense to the edge of the bank. Consequently there were smaller crowns intercepting light, and fewer reproductively active trees. Conversely, all of the stands with significantly greater proportions of reproductively active trees, had open or broken canopies, such that larger areas of crown were intercepting light. The positive correlation between the composite measure of disturbance and reproductive activity (Table 2.3) supports the contention that greater light penetration of the canopy stimulates cone production. Greater reproductive activity was also associated with lower altitudes (Table 2.5). This may reflect the association of Huon pine stands with the large open river systems such as the Gordon which are located at lower altitudes. These are subjected to disturbance from flooding and the often open nature of fringing stands allows more light penetration.

Figure 2.4. The total number of Huon pine plants (>1 m tall) in each diameter size class



These two trends which influence reproductive activity often act in opposite directions. Thus, stands with more light penetration and open or broken canopies, allowing for greater reproductive activity, are often those found on river fringes. These stands are also more likely to have a bias towards younger trees which will effectively reduce the proportion of reproductively active trees. Conversely stands away from river fringes are usually dominated by larger diameter, canopy trees (e.g. Greystone Bluff), but often also have relatively small crown sizes due to a more continuous canopy. Thus the two trends can counteract one another, possibly explaining why there is a close to average proportion of reproductively active trees in most stands.

Gender Expression

When all the data were pooled, there were overall, equal proportions of male and female Huon pine trees (Table 2.4). However, ten out of twenty five individual sites were dominated by one of the sexes (Table 2.4). Six sites were predominantly female (Pieman River, Riveaux Creek, Condominium Creek, Teepookana, Lake Vera, Franklin), and four were predominantly male (Stanley River, Spero, Huon River, Mt. Read). There was no observed difference in developmental expression of reproductive activity between female and male trees (Figure 2.3). The increasing proportion of reproductively active trees observed as diameter increases, occurs approximately equally in both sexes (Figure 2.3). Dominance of a gender type was not found to be correlated to geographic regions or proximity (Tables 2.3 & 2.5). There was no significant association of stands dominated by the same sex within a

thirty kilometre radius of one another. The ratio of males to females was apparently not associated with disturbance, stand structure, Huon pine density or climatic conditions (Tables 2.3 & 2.5). However, it was found that sites with similar vascular plant species composition also had similar proportions of trees of a particular gender (Table 2.3) which may indicate some environmental effect favouring one sex or differentially affecting gender expression.

Monoecious (bisexual) Huon pine trees were found in eleven out of twenty-five sites (Table 2.4). There was no consistency in the distribution of the sexes within monoecious trees. In some cases a tree was predominantly one sex with perhaps one limb of the other gender. In other cases, the tree was approximately evenly divided, but the genders still segregated and in other cases the sexes were completely interspersed on all branches. Overall monoecious trees represent 1% of all Huon pine plants greater than 1 metre tall and approximately 4% of those trees which were reproductively active during the study (Table 2.4). The Pieman River sites had significantly greater numbers of monoecious trees than other sites (Table 2.4). This locality was comprised of riverine and inland sites (Appendix 1). Most monoecious trees were found in the riverine sites.

Gender Distribution Within Stands

Two major patterns emerged when trees in transects were assessed for evidence of spatial autocorrelation of reproductivity and gender types. Firstly reproductively active and inactive trees were negatively autocorrelated, suggesting clumps of either reproductively active or inactive trees (Table 2.6). This result was found in almost all sites analysed (Table 2.6). The non-significant exceptions occurred where there were small numbers of reproductive individuals in the stand. Male and female trees also tend not to occur together (Table 2.6). All of the sites with non-significant results, (except Junction Creek), either had low numbers of reproductively active trees or low numbers of one sex, and therefore may have had too few trees of the opposite sex to produce significant results. When combined, these results suggest that sexually unlike trees tend to be separated from each other, since there is an overall negative spatial autocorrelation between unlike trees (Table 2.6).

There was however evidence for a significant positive association of like trees within a ten metre radius (Table 2.6). For example, non-reproductively active trees were more likely to occur as neighbours or to be clumped. This was so for all sites surveyed (Table 2.6). As stands were mostly comprised of non reproductively active trees (Table 2.4).

Table 2.6. Summary of spatial autocorrelation results over all Huon pine sites studied. The results of each reproductive pair combination are pooled over all study sites and given as percentages of the total number of each type of pair combination. P< 0.05; nonrep = trees not reproductively active.

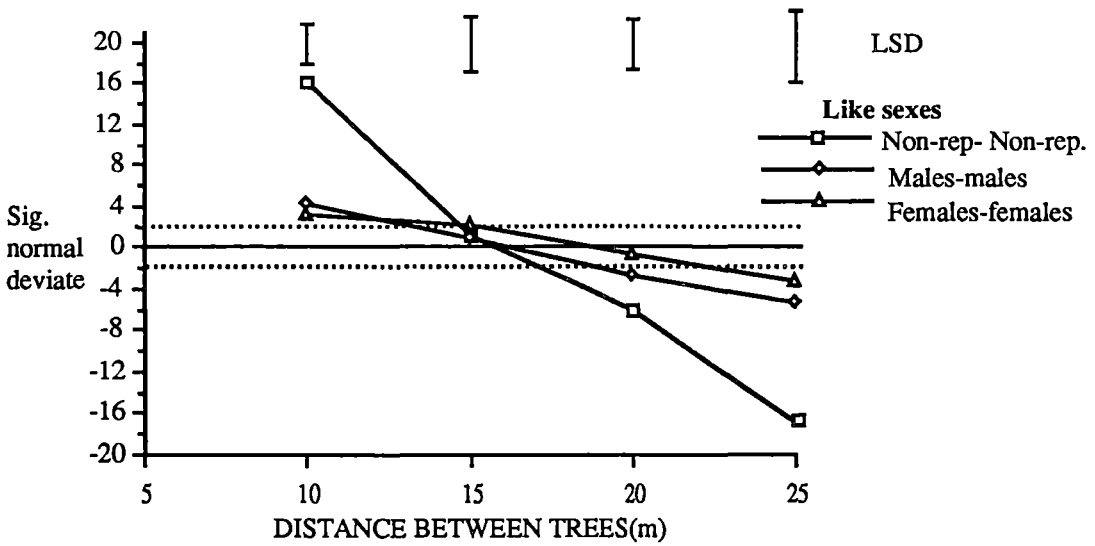
Spatial autocorrelation between trees within a 10 metre radius

Pairs	%sig +ve	%non sig	%sig-ve
Like			
male-male	60	40	0
female-female	47	53	0
nonrep-nonrep	100	0	0
Unlike			
male-female	0	40	60
male-nonrep	0	13	87
female-nonrep	0	7	93

It is not surprising therefore that they would tend to occur as neighbours. Both male and female trees however, were also most likely to be found alongside other trees of the same sex in over half of the study sites tested (Table 2.6). Again the non-significant results were nearly all attributable to lower numbers of individuals. The exceptions were non-significant numbers of male-male associations at Teepookana and non-significant female-female joins at Pine Creek and Picton River. The significant positive association of male trees with one another counters the possibility of this observed pattern being solely due to maternal effects.

The associations between like trees and between unlike trees were observed with increasing distance between neighbouring trees (Figure 2.6). The negative association between trees of different gender or reproductive status remained, whereas the positive association between trees of the same type diminished with increasing distance (Figure 2.6). Epperson and Clegg (1986), have suggested that the point where the correlogram crosses the origin (non-significant result) may represent the patch size. If this is accepted, then there is a consistent patch size of approximately fifteen metres radius for trees of like reproductive expression amongst sites (Figure 2.6). The observed negative correlations at larger distances, could be due to several possible effects. However, because of the long narrow nature of the stands there may be too few neighbour (joined) trees at larger distances, which may have resulted in statistical artifacts. This effect has been noted by other authors (Epperson and Clegg 1986).

Figure 2.6. Autocorrelation of like reproductive status associations correllogram. The significant normal deviate scores (SND) for each pair category were averaged over all sites. The correllogram illustrates the change in the mean significant normal deviate for each like reproductive status pair with increasing distances between pairs of trees. The dotted lines indicate the p<0.05 significance level for SND values. The Least significant difference (LSD) between the pooled SND values is indicated by the error bars at each distance class.



DISCUSSION

Huon pine populations have been shown to vary in their stand size structure, in both this study and by Gibson and Brown (1991). Huon pine diameter has been shown to be linearly correlated with age (Francey *et al.* 1984). However, diameter increases ranging from 0.3-2 mm annually have been recorded (Gibson and Brown 1991). Thus, the assumption that size reflects age has some justification in this species. Growth suppression will alter the linear relationship between diameter and age. It is more likely to occur in dense stands of subcanopy height saplings, and in old mature trees (Ogden 1985, Gibson and Brown 1991). Suppression in the understory, particularly in rainforest trees, can last for a considerable number of years. It has been suggested therefore that size structure itself may be more relevant to understanding the ecology of a species (Ogden 1985). The present study surveyed a greater number and variety of sites than that of Gibson and Brown (1991), and the range of stand structure and regeneration patterns found were consequently greater and more complex.

Stand structure was found to be correlated with measures of stand disturbance (Table 2.3). Gibson and Brown (1991) suggested that disturbance was required for Huon pine regeneration, even though Read (1985) demonstrated it was shade tolerant. There was evidence of some regeneration in all sites regardless of their structure, therefore these results do not infer that disturbance is required for regeneration.

However, disturbance will affect the nature of regeneration in Huon pine. Gibson and Brown (1991) correlated stand structure to height above river level. They found more pulsed patterns of regeneration evident in higher stands and negative exponential growth closer to the river. However, this pattern does not hold when more sites are studied (Figure 2.2). Flooding is a major source of disturbance in Huon pine stands on the big rivers. Major rivers will therefore correlate with higher disturbance levels. The same level of disturbance is unlikely to occur in Huon pine stands growing on small creeks. This may partially explain the differences between these results and those of Gibson and Brown (1991).

The results of the present study suggest that both recruitment and mortality in Huon pine populations vary depending on site conditions. Thus growth models which predict constant recruitment and mortality, such as the negative exponential growth model, may not be appropriate (Hett and Loukes 1976). Hett and Loukes (1976) found that models which predict sinusoidal fluctuations in age distributions were more realistic for long lived species in undisturbed forests. Furthermore populations which are undergoing self thinning are less likely to allow new recruitment since sites are overstocked already (Ogden 1985). This theory has been applied to dense 'ricker' stands of New Zealand podocarps (Stewart and Rose 1989). The results of Gibson and Brown (1991), indicated self thinning in some Huon pine populations, since, as the size of trees increased, the average distance to nearest neighbour also increased. Therefore high levels of new recruitment may not be expected in all sites.

The density within Huon pine stands was correlated with geographical location (Table 2.3) and was found to be greatest in stands in the south east (Table 2.5). Since density was measured as Huon pine trees (dominant stem) per square metre, the higher density of these stands reflects the generally smaller trees found at these sites. The south-eastern sites occur at the drier extreme of the Huon pine distribution. Many of these sites, according to Gibson *et al.* (1991), lie outside the Tasmanian rainforest range. The eastern sites are found as fringing riparian fragments. They are usually associated with other rainforest species, but often have a high sclerophyllous component in the community. These sites tend to have lower rainfall but greater soil fertility than in other parts of the Huon pine distribution (Gibson *et al.* 1991). These stands tend to be small in size and though they may be persistent, the smaller size of mature trees suggests they probably have a higher turnover rate of trees. Disturbances, particularly to the surrounding drier vegetation, would no doubt contribute to this turnover. For example, fires would periodically burn up to the boundaries and encroach on the stands, for example at Riveaux Creek, Anne River and Condominium Creek. More recently clearfelling has also occured up to the

boundaries of some stands (e.g. Riveaux Creek). The stands maintain themselves by virtue of the moist microclimate created around the rivers and creeks and by having their 'feet' in water.

Stands of trees in more stable higher rainfall areas, such as Greystone Bluff, Teepookana and Sr. John Falls, which are away from river fringes, have grown undisturbed except for wind throw. However, poor soils may have reduced the stocking capacity of these sites, and the larger root systems of mature trees may limit the establishment of younger trees. Occasional wind-throw creates canopy gaps, enabling suppressed seedlings to grow, and providing root-free soil. Since Huon pine is so long lived, few replacements are needed for population maintenance over very long time scales. Ogden *et al.* (1987) calculated for *Agathis australis* in New Zealand, that canopy gaps due to tree fall could be expected, such that all parts of one hectare will have a gap over a 500 year period. He speculated that if such gaps were suitable for colonisation for about fifty years, then approximately three gaps per hectare would be continuously available for recruitment. While the details will be different, a similar scenario may be postulated for Huon pine. In contrast to the New Zealand conifers (Stewart and Rose 1989, Ogden 1985) and the Tasmanian *Athrotaxis* species (Cullen 1987) Huon pine does not show an ability to regenerate after mass disturbance events. The desiccating conditions resulting from mass disturbance may kill off most seedling regeneration until a moss cover is established, and faster growing species are likely to out-compete the slow growing Huon pine seedlings.

Disturbance was also correlated with reproductive activity (Table 2.3). The nature of disturbance at most sites was not severe and tended to result in broken canopies and small light gaps. In most cases where logging had occurred it was selective and did not use heavy machinery. There were two examples where disturbance was on a larger scale, at Teepookana and on the Gordon River banks. Teepookana stands have been nearly clearfelled in places, with tracks and roads criss-crossing within them. Many trees now stand isolated above a shrub layer of regrowth. Their crowns are severely reduced, apparently due to the desiccating effect of wind or due to soil compaction. Whilst some trees appear to be dying, others are producing cones, but the small crown sizes reduces both the output and the potential dispersal for seed (Chapter 1) . There was evidence of some seedling success in patchy locations, but Gibson (1987) reported poor evidence of regeneration on this site. High levels of reproductive activity were recorded along the banks of the Gordon River, where Huon pine occurs as fringing vegetation. It has a patchy but almost continuous distribution along the length of the river. However, most of this seed will be washed

out to sea, because the trees overhang the banks. More seriously, the banks are being eroded by wash from tourist boats, ironically transporting tourists who wish to view Huon pine! Many Huon pine trees are being lost as the banks collapse. This bank instability will also reduce the chance of successful Huon pine regeneration. Huon pines in the past, here, as in most riparian stands, usually stabilised banks due to their large root systems.

The effects of disturbance on reproduction are probably mostly associated with light availability to the crowns of Huon pine trees. Light has been observed to stimulate reproductive expression in many plants and flowers and cones are commonly seen capping the outer surface of plants (Sedgley and Griffin 1989). Light is thought to act by altering the balance of hormones in the shoot tissue such that initiation of the development of the reproductive organs can occur (Ross and Pharis 1987, Sedgley and Griffin 1989).

To some extent growth status may affect the expression of reproductive activity (Ross and Pharis 1987). Whether a tree puts energy into cone production may be affected by factors determining its growth (Bell 1985, Sedgely and Griffin 1989). For example large dominant trees may no longer require as much energy for growth and their energy is better spent on reproduction (Linhart and Mitton 1985), whereas younger trees may put their energy towards attaining maximum size. This trend was observed in this study since as trees became larger (older), they were more likely to be reproductively active (Figure 2.3). However, in some species, reproduction has been observed to reach a peak at some middle age, after which it tends to be reduced in older age classes (e.g. Falinski 1980). Therefore the size (age) structure will affect the level of reproductive activity expressed in a population. Disturbance affects both size structure and reproductive activity through more open canopies. However, more disturbed sites often have proportionately more small sized trees and fewer larger more reproductively active trees. A size structure biased towards large trees will often have less light penetration of its canopy. If larger trees reproduce more regularly, then a few trees may contribute disproportionately to the gene pool for future generations (Sakai and Oden 1983). At Riveaux creek the same large trees have been reproductively active over a few successive seasons. If there is more competition for energy between vegetative growth and cone production in younger trees, it may be expected that smaller trees may be more variable in their reproductive expression from season to season. Trees which have their size suppressed due to stand density, but which get sufficient light to stimulate cone production, may direct energy into cone production rather than vegetative growth. However, their capacity to do this will be limited by resources such as nutrients. In

some species sexual reproduction is profuse after stressful periods such as droughts. Indeed horticultural practices use artificial stresses such as girdling and savage pruning to induce flowering in trees (Sedgley and Griffin 1989, Ross and Pharis 1987). Cone production in older Huon pine trees is usually extensive with terminal branchlets of the whole crown covered in cones. This limits vegetative shoot growth in the crown. On cone senescence in both sexes, the terminal portion of the shoot is shed along with the old cones. However, on smaller trees which are still actively growing, no apical dominance was observed and vegetative shoots were observed to grow past the developing cones. This suggests that more vigorous Huon pine trees in favourable conditions may achieve both vegetative growth and cone production in a season.

It has been argued that the energy cost of producing female reproductive organs is greater than the cost of production of males, because of the additional energy requirement to produce seed (e.g. Freeman *et al.* 1976, Sedgley and Griffin 1989, Lloyd and Webb 1977). It is unclear if this is the case in Huon pine. The cone size is similar for both sexes however the male cone takes several months longer to mature than the female cone does to become receptive, but seed development can take an additional five to twelve months to seedfall. Female cones however, are not produced every year. Male cones are produced more regularly, though not necessarily every year (pers. obs.) which is consistent with observations in other species (Lloyd and Webb 1977, Freeman *et al.* 1976). Given that male trees seem to produce cones more often, it is unclear whether the overall energy costs between the sexes differs. However, the duration and timing of energy costs does differ and could be significant.

The differing initiation cycles for male and female reproduction, and the differing times of cone initiation, suggest that male and female Huon pine trees are responding to different environmental stimuli for cone initiation. This has been suggested to be the case for many other species (Ross and Pharis 1987). It was observed that initiation of female cone development takes place later than that for males, a phenomenon that has been observed in other species (Sedgley and Griffin 1989, Ross and Pharis 1987). It is critical that the timing of maturation of the cones of each sex coincides. For example, slightly delayed female cone production at Riveaux Creek meant that pollen shedding occured approximately two weeks before female cones were receptive and the result was no seed set at all! If cone initiation and development is environmentally regulated then changes in climate, such as occurred during the break-up of Gondwana, may have affected reproduction success in this species.

It has been suggested that in many plant species, male plants tend to survive better in harsher , more stressful environments, whereas females predominate in more mesic or fertile sites (Freeman *et al.* 1976, Sakai and Oden 1983, Lloyd and Bawa 1984). Indeed Falinski (1980) showed that male/female ratios in several conifers changed with successional status of the populations on old fields. On harsh newly-colonised sites males predominated, equal ratios were found on older sites and on still older sites females predominated. Such trends are often explained by both the additional energy and nutrient requirements for seed production, the need for longer periods of good conditions as the seed develops (Lloyd and Bawa 1984). In the present study the association of sites with similar proportions of females and sites with similar species compositions (Table 2.3) was the only correlation of those tested which related to gender balance. This result suggests that site variability which affects species composition may also affect the gender proportions of the population. Gibson *et al.* (1991) found that floristic variation in Huon pine populations was correlated with climate and geological gradients. In the present study, climate was associated with species composition, coinciding with Gibson *et al.'s* (1991) results, but the proportion of females was not. This may suggest that geology has an effect through soil characteristics, on female selection or expression. Unfortunately soil measurements were not included in this study. These results show some partitioning of gender between environments. However, microsite conditions may also affect the expression and survival of female trees.

In some species, density has been correlated with reproductive activity and gender type (Falinski 1980). This has not been found in this study (Table 2.5). Other studies have found size or age differentiation between the sexes (e.g. Hibbs and Fischer 1979, Heslop-Harrison 1957, Falinski 1980, Clark and Orton 1967). Some studies have found strong size correlations to be particularly so for females, and have suggested it may be advantageous to attain greater sizes to increase chances of pollination (Falinski 1980, Nakumura *et al.* 1989). Alternatively, it has been argued that it may be advantageous for males to remain smaller as their pollen will have a relatively greater chance of success (Nakumura *et al.* 1989). There is no evidence for this to be so in Huon pine populations as both sexes appear to have equivalent developmental expression, although the largest tree found in the study was female!

Most other studies have, like this one, found equal proportions of male and female trees (Lloyd and Bawa 1984) (Table 2.4). However, unequal sex proportions have been found in some species (e.g. Lloyd and Bawa 1984, Freeman *et al.* 1976, Melampy and Howe 1977). Other studies have also found sex ratios for particular

species varying between sites (Lloyd and Bawa 1984) as was observed in this study (Table 2.4). The observed sex ratio in a population may be altered if more of one sex is stimulated to be reproductively active in any one season, or if trees change their gender expression in response to particular environmental stimuli (Sedgley and Griffin 1989, Ross and Pharis 1987). Sex change has been observed in some species (Freeman *et al.* 1976, Primack and McCall 1986). In one of the few studies that have monitored trees in natural populations for their constancy, Primack and Macall (1986) found that predominantly male or predominantly female trees remained constant, but that indeterminant (bisexual) trees were more variable over time. The constancy of Huon pine trees during their lifetimes is unknown, though observations of garden specimens suggest gender to be constant. Huon pine bisexual trees may alter their gender expression. However, they are unlikely to significantly alter the sex ratios of most populations since they occur with such small frequencies (Table 2.4). Their presence does however suggest, that there may be environmental as well as genetic control of sex determination in Huon pine populations. Sex ratio may also be altered by vegetative reproduction. Either particular clones may be more successful on a site, or stands may have developed vegetatively from founders of mostly one gender. Huon pine is known to reproduce vigorously by vegetative means and this is quite likely to have affected its sex ratios. Mt. Read is an extreme example where it appears the stand has developed primarily vegetatively (Chapter 3), and to date only males have been recorded in this stand (Table 2.4 and pers. comm. M. Peterson). Other studies have found males to be favoured at higher altitudes (Freeman *et al.* 1976) is it a coincidence that Mt. Read is the highest altitude Huon pine stand known?

This study of reproductive expression in natural populations, has been more extensive in its sampling of populations than most others (e.g. Bawa and Opler 1977, Primack and McCall 1986, Lloyd and Bawa 1984, Melampy and Howe 1977). By sampling the range of this species the results are less site specific. Few other studies have investigated the spatial distribution of gender types within populations (Sakai and Oden 1983). Some have investigated microsite preferences of gender between sites (e.g. Freeman *et al.* 1976). Bawa and Opler (1977) found random distributions of male and female trees in tropical angiosperms. Sakai and Oden (1983) found like sexes to be associated, but correlated to size, which was clumped, and found sex to be randomly distributed within patches of similar sized trees. Huon pine showed a consistent spatial pattern in all sites of like sex association at short distances. This clumping of like sex may reflect the average area a vegetative clone occupies in stands and was remarkably consistent between sites (Figure 2.6). Clumping was also found in the reproductive expression of trees in stands. Reproductively active trees

were positively associated with each other and tended not to have non-reproductive neighbours (Table 2.6). This separation of reproductively active and non-reproductively active trees did not diminish with distance (Figure 2.6), which suggests influences other than purely vegetative spread. Gibson and Brown (1991) also showed Huon pine trees to be physically clumped in their spatial distribution.

Vegetative reproduction can overcome the influences of microhabitat patchiness on sex expression (Lloyd and Bawa 1984). Patchiness, for example in soil moisture, fertility, pH, and light availability may, as noted previously, affect the distribution of reproductive activity or the gender of trees in populations (Freeman *et al.* 1976, Falinski 1980). The nonrandom distribution of reproductively active trees and of gender will tend to reduce the effective breeding population, especially if the same few trees produce each season, and lead to non random mating within populations. These effects will severely limit the potential of the sexual reproductive system to increase genetic variability within populations.

Huon pine is a species which can reproduce vegetatively, and which may at times have poor recruitment via seedlings. Vegetative reproduction can be seen as an adaptation which bypasses the vulnerability of the seedling phase (Johnson and Lacey 1983). They have suggested that vegetative reproduction is common in Australian southern temperate rainforest species, and is a survival strategy. There is no doubt that vegetative reproduction enables Huon pine populations to persist for great lengths of time (Francey *et al.* 1984). However, it may reduce the genetic variability of populations and therefore the exchange and recombination of genes that sexual reproduction allows. This may be important for long term survival of a species, both for adaptation to changing environments and for disease resistance (Bremmerman 1985).

The understanding of the relationship of sexual versus vegetative reproduction is fundamental to the understanding of the evolutionary process. There are many theories pertaining to the selection for, and maintenance of, sexual reproduction within species and populations (Leigh 1970, Bell 1985, Koella 1988). It has generally been thought that the advent of separate sexes was an adaptation to maintain genetic diversity (Bawa 1980, Bell 1985). While this may not have been the selective force producing this state (Bremmerman 1985), it has had this effect. The obligate outcrossing associated with dioecy is a mechanism which maintains recombination and variability. Such Recombination and variability is necessary perhaps for the long term survival of vegetatively reproducing species, such as Huon pine. However, the limited reproductive expression in Huon pine populations and the

non-random distribution of those trees, will mean nonrandom mating in populations, thereby reducing the potential for genetic recombinations. Thus while most pollen reaching female cones will be from local sources, that which comes in from other populations may be important in maintaining gene flow between populations and in reducing the strong trends for drift and inbreeding in populations.

CHAPTER 3 :

Population genetic analysis of Huon pine sites.

INTRODUCTION

Relatively few studies of population genetics have been undertaken on very long-lived species (e.g. Finns and Libby 1982). It is important that such studies are undertaken to confirm the applicability of trends observed in other species (Hamrick and Godt 1989). Huon pine is very slow growing (Francey *et al.* 1984) and amongst the longest-lived of trees. Few population genetic studies have been undertaken on species within the Podocarpaceae (Hawkins and Sweet 1989, Hasse 1992b, Billington 1991), yet this family dominates southern hemisphere conifers and contains some of the longest-lived trees. The proliferation of genetic studies on northern hemisphere trees, especially the Pineaceae, may have biased our genetic expectations for long-lived species and conifers, since, for example, many aspects of their ecology and reproductive biology differ from other gymnosperm families.

Several authors have emphasised the need to integrate population genetic studies with ecological, demographic and reproductive studies, since genetic variation is influenced by the biology of a species and its responses to the environment (Lande 1988, Antonovics 1976, Levin 1978). However, relatively few have done so (e.g. Antonovics *et al.* 1988, Linhart *et al.* 1979, Finns and Libby 1982, Murawski and Hamrick 1991, Epperson and Clegg 1986). Attempts have been made to relate species ecology and life history characteristics to genetic structure and variability using surveys of available literature (e.g. Hamrick and Godt 1989, Loveless and Hamrick 1984). When demographic and biological studies are undertaken in conjunction with population genetics a greater understanding of the processes which affect species' populations and hence better strategies for conservation can be determined (Lande 1988).

Huon pine is predominantly dioecious and an obligate outcrosser. Its winged pollen may potentially be dispersed long distances. Its seed is poorly dispersed by gravity, but highly adapted to dispersal by water with no apparent reduction in germination due to immersion (Chapter 1). There is generally low germination success compared to seed output (Chapter 1). Huon pine is found mostly in subdivided populations as small stands in rainforest associated with the river systems of the south west of

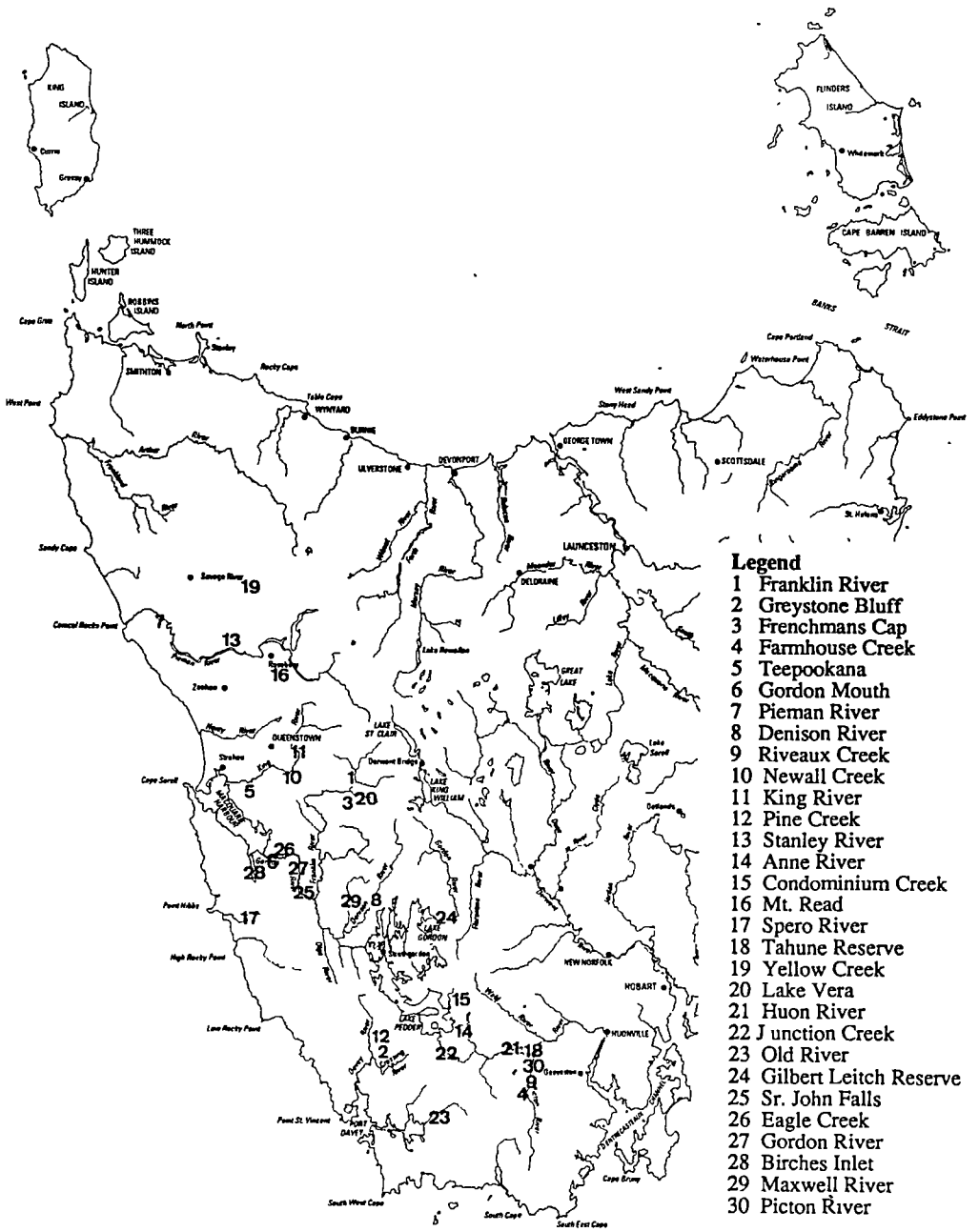
Tasmania (Davies 1983). Even within larger stands Gibson and Brown (1991) found clumping of trees. Huon pine populations are thought to have retracted to protected refugia during the Last Glacial (Macphail 1979). Disturbances such as burning, together with the restricted dispersal and slow growth of Huon pine, have probably restricted its potential expansion since the Last Glacial (Gibson and Brown 1991). Light availability affects the size structure and also the proportion of reproductively active trees in Huon pine stands (Chapter 2). The largest trees may contribute disproportionately to reproductive output, thus the structure of a stand may affect the genetic composition. Only a small proportion of trees may be reproductively active in a season and these are non-randomly distributed throughout stands (Chapter 2). Huon pine also reproduces vegetatively. Therefore conditions of panmixia do not appear to be met in Huon pine stands. Huon pines' subdivided population structure, its distribution over a variety of habitats, its ecology, reproductive biology and life history traits, may have impacts on its genetic structure at many levels.

Isozyme analysis was used to investigate the distribution and amount of genetic diversity within the species, among its populations, and within stands. The study determined whether or not stands complied with Hardy-Weinberg expectations or if they are effectively inbred, and what effect this has on patterns of genetic variability and survival of the species. Explanations for the patterns of breeding and variability observed, were sought by comparison with biological and ecological data on reproduction, stand structure and density, species composition, climate and geography. Detailed mapping of Huon pine stands enabled the analysis of genetic spatial structure within stands to be compared with reproductive spatial structure. In addition, the utilisation of information on Huon pines' biology and ecology, geographic distribution and fossil history was used to assist in the interpretation of the data.

FIELD METHODS

Material was collected from 30 locations across the ecological and geographical range of Huon pine (Figure 3.1). Some locations consisted of several collection sites. These included the Pieman River which consisted of seven sites, the Franklin River, consisting of 5 sites and the Gordon mouth consisting of three sites. The Denison and Spero River collections consisted of sites where transects were undertaken off the river bank as well as riverside collections. The Teepookana location was composed of several transects in close proximity. When appropriate, data were excluded, kept separate or grouped for analysis.

Figure 3.1 The locations of the Huon pine isozyme study sites.



Samples were collected from Huon pines of all sizes, including very small seedlings. They were assigned a unique number, and their positions were mapped relative to a transect line. The height and diameter of each tree was recorded and where possible its gender determined (see Chapter 2). Species lists were compiled for twenty six sites. At some sites, due to time limitations, not all transect data were obtained (see Chapter 2). At four sites (Yellow Creek, Old, Maxwell, Franklin) samples were obtained randomly. These were cases where fortuitous circumstances allowed sampling of quite inaccessible sites to be undertaken, often by enthusiastic co-workers, but where time was limiting.

Shoot material was used for electrophoretic analysis since seed production is infrequent, germination very slow and unreliable (see Chapter 1), and since adult material gives the current genetic composition of stands. Samples were placed in individual sealed plastic bags. They were kept refrigerated on return from the field (within a few days) and were frozen (-20°C) and then freeze dried as soon as possible given the space limitations of the freeze dryer (within weeks). The freeze dried samples were then stored in sealed containers in a freezer (-20°C) until used.

LABORATORY METHODS

The grinding and extraction of enzymes from leaves for electrophoresis of enzymes, must be done quickly to prevent enzyme denaturation. For population studies, the techniques must also be suitable for processing large numbers of samples. The extraction method used was an adaptation of the methods of several authors (Gan *et al.* 1981, Neal *et al.* 1984, Cheliak and Pitel 1984). Each sample was ground in liquid nitrogen with a mortar and pestle, to a coarse powder. Extraction buffer was mixed with the powder (Table 3.1), and this mixture was squeezed through a coarse filter and absorbed onto filter paper wicks (Whatman number 1). These were kept on ice until loaded onto the gel. The extraction buffer used for woody plants is complex because it must counteract the effects of compounds such as phenols and tannins, which are present in leaves and which, in the ruptured cell, act to bind to or otherwise inactivate enzymes. The buffers contain a range of additives which act to isolate and stabilise enzymes in a buffered solution (Stahman 1963, Loomis 1974). These include antioxidants such as ascorbic acid and sodium metabisulphite which form complexes with phenolics and quinones and thus protect enzymes (Loomis 1974). Metal chelating compounds such as DIECA (Diethylcarbamide) and EDTA (Ethylene Diamino Tetra Acetic acid) competitively bind metal ions which might otherwise inactivate enzymes (Loomis 1974). Sucrose reduces the precipitation of

plant proteins. Other chemicals include absorbents such as PVP (Loomis *et al.*1979) as well as Borax and Albumin which bind to phenols and so prevent enzymes from doing so (Stahman 1963, Loomis 1974).

Table 3:1 Electrophoresis Extraction Buffer

(modified from Cheliak and Pitel 1975)

STOCK SOLUTION		
	0.1M	Phosphate buffer pH 6.8
	100g/L	Sucrose
	70g/L	Polyvinyl pyrrolidone (PVP) (40000MW)
	10g/L	PVP (360000MW)
per 50 ml of stock solution add:		
	50 mg	Ascorbic acid (Na salt)
	85 mg	EDTA (di Na)
	300 mg	NaS_2O_5
	400 mg	Borax
	500 mg	Albumin (bovine serum)
	225 mg	DIECA
	50 mg	Dithiothrietol (DTT)
	10 mg	NADP
	20 mg	NAD

Samples were run on 12.5% starch gels using the methods described by Conkle *et al.* (1982). Gels were run under constant current conditions of 50 mA for 6.5 hours. A discontinuous Lithium hydroxide, Tris buffer system was selected. This gave the best resolution of the bands (Table 3.2) after four standard buffers had been tested.

Table 3.2 Electrophoresis Gel and Tank Buffers

Lithium hydroxide discontinuous system (Brewbaker *et al.* 1968)

GEL BUFFER pH 8.2	0.065 M	Tris
	0.01 M	Citric acid
TANK BUFFER pH 8.5	0.05M	LiOH
	0.19 M	Boric acid

The gel is made with a 9:1 mixture of gel buffer: tank buffer

Initially a wide variety of enzyme specific stains were screened to investigate those which produced clearly readable bands (Isocitric dehydrogenase, Malate dehydrogenase, Fumarase, Glycerate dehydrogenase, 6-Phosphogluconate dehydrogenase, Phosphoglucose isomerase, Glucose-6-phosphate dehydrogenase, Glutamate dehydrogenase, Esterase, Aspartate aminotransferase, Malic enzyme, Phosphoglucose mutase, Acid phosphatase). Most staining systems gave poor results. The following stains were chosen for further use: Isocitric dehydrogenase (IDH); Peroxidase (PER), Glucose-6-phosphate dehydrogenase (G6PDH); 6-Phosphogluconate dehydrogenase (6PG); and Aspartate aminotransferase (AAT). Stain recipes are given in Table 3.3.

Table 3.3 Recipes for Enzyme Stains

IDH (Isocitrate Dehydrogenase) (E.C.1.1.1.42) (Shaw and Prasad 1970)

135ml	H_2O
10ml 1.0M	Tris pH 8.0
1ml	$MgCl_2$ (10%)
50mg	(Na) Isocitrate
32mg	MTT
15mg	PMS
10mg	NADP

6PG (6-Phosphogluconate Dehydrogenase) (E.C. 1.1.1.44) (Brown *et al.* 1978)

127ml	H_2O
10ml 1.0M	Tris pH 8.0
40mg	6-Phosphogluconic acid
32mg	MTT
15mg	PMS
10mg	NADP

G6PDH (Glucose-6-Phosphate Dehydrogenase) (E.C.1.1.1.49) (Shaw and Prasad 1970)

135ml	H_2O
15ml	Tris pH 8.0
50mg	(Na) Glucose-6-phosphate
32mg	MTT
15mg	PMS
10mg	NADP
10mg	(Na) Cyanide

PER (Peroxidase) (E.C.1.11.1.7) (Shaw and Prasad 1970)

150ml	0.2M Sodium acetate buffer pH 4.9
100mg	$CaCl_2$
1.5ml	1% H_2O_2 (dissolved in 5ml Dimethylformamide)

AAT (Aspartate aminotransferase) (E.C.2.6.1.1) (Brown *et al.* 1978)

4ml	0.2M Phosphate buffer pH 4.9
250mg	PVP
0.2ml	(0.05%) Pyridoxal-5-phosphate
1ml	(1.06%) a-Ketoglutaric acid
1ml	(4.56%) Aspartic acid
50mg	Fast blue BB salt (in 2ml H_2O)

STATISTICAL METHODS

The BIOSYS-1 statistical package (Swofford and Selander 1981) was used to analyse the IDH, 6PG and G6PDH genetic data. Allelic frequencies at each site were calculated and used to compute for each site: the mean number of alleles per locus (A), the mean percentage of polymorphic loci (P), the observed mean heterozygosity (Ho), and the expected mean heterozygosity under Hardy-Weinberg conditions (He) using Levene's (1949) correction for small sample sizes. Each site was tested for conformance of genotypic frequencies to those expected under Hardy-Weinberg

equilibrium using a Chi-square goodness of fit test (sig. level $P<0.05$). The degree of inbreeding in populations was assessed by calculation of genotypic fixation indices (F) (Wright 1965) at each polymorphic locus where, F=(He - Ho)/He.

Genetic diversity among all sites was calculated using FST (Wright 1965) which is equivalent to Nei's (1973) GST (Nei 1977). Although developed from fixation indices this measure uses expected heterozygosity levels in its calculations and is thus independent of inbreeding. Sites were grouped according to the catchment system they were associated with (Table 3.4) and Wright's (1978) hierarchical F statistics were used to analyse the partitioning of diversity and variance in this hierarchy (i.e. between all sites, between catchments and between sites within catchments). The significance of intersite heterogeneity in allelic frequencies was evaluated by using a heterogeneity Chi-square test (Workman and Niswander 1970).

Table 3.4. The Heirarchy of Huon pine sites based on catchment that was used for genetic analysis.

CATCHMENT	SITES	CATCHMENT	SITES
Gordon	Lake Vera	**King**	King
	Frenchmans Cap		Newall
	Franklin Upper		Teepookana
	Franklin Irenabys		
	Franklin Lower	**Davey**	Pine
	Great Ravine		Greystone Bluff
	Gordon River		Old
	Gordon River Mouth		
	Sr. John Falls	**Pieman**	Pieman
	Birches Inlet		Stanley
	Denison		Yellow
	Maxwell		
	Gilbert Leitch	**Spero**	Spero
Huon	Huon	**Mt. Read**	Mt. Read
	Tahune		
	Picton		
	Riveaux		
	Farmhouse		
	Anne		
	Condominium		
	Junction		

Matrices of genetic similarity and distance between pairs of sites, were generated using the co-efficients of Rogers (1972), modified by Wright (1978), and of Nei (1978), across all loci. Matrices were also calculated to measure genetic distance between catchments. An Unweighted Pair Group Method with Arithmetic averaging (UPGMA) (Sneath and Sokal 1973) cluster analysis and a distance Wagner analysis (Farris 1972), were performed on these matrices to produce dendrograms of the genetic relationships between sites. The goodness of fit of the dendrograms was

assessed using several measures, including co-phenetic correlation (Sneath and Sokal 1973), the F value of Prager and Wilson (1976), the 'f' of Farris (1972) and the percentage standard deviation (Fitch and Margoliash 1967). The dendrograms produced by different methods and measures were compared and the cluster with the best fit was used for further interpretation since similar groupings were obtained by all methods.

As the peroxidase zymograms could not be confidently interpreted as allelic frequencies, they were not included in the above analyses. However, the presence/absence data recorded for each band was converted to band frequencies at each site and analysed by an analysis of deviance test using GENSTAT:V (Rothamstead Experimental station 1984). The null hypothesis was that there were differences between band frequencies, but not for any particular band between sites. Therefore, the expected value for each band, in each site, was the average frequency of each band over all the sites. The significance of any deviation in band frequencies was then determined. The frequency data was also subjected to a UPGMA cluster analysis (Sneath and Sokal 1973) using Euclidean distance on SAS ® (SAS inst. inc 1990) and a dendrogram was produced.

The genetic information from allelic frequencies and peroxidase band frequencies for each site was pooled to generate a combined measure of genetic distance using Euclidean distance and clustered using a UPGMA on the PATN statistical package (Belbin 1991).

Comparison of Distance Measures

Roger's (1972) (modified by Wright 1978) genetic distance was compared with a variety of other distance measures between sites using the Mantels test (1967) which was undertaken on a program based on that published by Manley (1985). Dissimilarity matrices were generated for several other variables. Squared Euclidean distance was used to generate distance matrices from geographic co-ordinates, percentage of reproductively active trees, proportion of female trees, density of Huon pine trees and seedlings, a disturbance measure combining several variables (see Chapter 2), a composite structure variable (see Chapter 2) and a composite climate measure generated from the four PCA/BIOCLIM synthetic variables (see Chapter 2). The PATN statistical package (Belbin 1991) was used to generate these distance matrices. The DECODA statistical package was used to calculate a dissimilarity matrix based on species presence or absence using the Bray-Curtis distance measure

(Minchin 1990). The Mantels test was used as a test of association between pairs of dissimilarity matrices using regression (Manley 1985).

Correlations

The sites sampled were scored for a number of structural and ecological characters, a summary of which is given in Table 3.5, as well as several genetic parameters. The genetic parameters used were as follows; frequencies of the allozymes, IDH (A), 6PG (A) and (C), G6PDH (A) and (C), average heterozygosity, IDH fixation index (Wright 1965), and the combined number of allozymes and PER bands differing significantly from their average frequencies. These variables were then analysed using Spearman's rank correlation on SAS® (SAS Inst. Inc. 1990).

Table 3.5. Variables used in Spearmans Rank correlation matrix

Variable	Description
Easting	Universal grid reference Easting (100 metre units)
Northing	Universal grid reference Northing (100 metre units)
Altitude	Altitude (metres)
Climate1	Climatic principal component 1 generated from PCA analysis of BIOCLIM generated synthetic climate profiles.
Climate2	Climatic principal component 2 " "
Climate3	Climatic principal component 3 " "
Density	Density, the number of Huon pine trees per square metre.
Seedling Density	Seedling density, the average number of Huon pine seedlings per square metre (all Huon pine plants less than one metre tall were classed as seedlings)
Prop Females	The proportion of dioecious reproductively active Huon pine trees which were female.
Reproductive	The proportion of reproductively active Huon pine trees.
IDH A	Frequency of IDH allele A.
6PG A	Frequency of 6PG allele A.
6PG C	Frequency of 6PG allele C.
G6PDH A	Frequency of G6PDH allele A.
G6PDH C	Frequency of G6PDH allele C.
Heterozygosity	The observed average proportion of heterozygotes.
IDH Fixation	The IDH fixation index F (Wright 1965).
Significant bands	The combined number of PER bands and allozymes which differed significantly from overall average proportions

Spatial Autocorrelation

The data were analysed for spatial autocorrelation (see Chapter 2). Trees within transects were analysed for spatial autocorrelation of their IDH genotypes. Allelic variation was not sufficient in the other enzymes for the analysis to be undertaken on the spatial distribution of their genotypes. The actual coordinates of Huon pine trees within sites were used for the analysis. These formed an irregular lattice of sample points. Pairs of trees were considered to be neighbours within a radius of ten metres.

Ten metres was estimated as the approximate area of influence of any one tree, based on field observations and seed dispersal (Chapter 1). The distance also took into account the tree density (Chapter 2), such that there were likely to be enough neighbouring trees to give statistically meaningful results. The variation in spatial autocorrelation that occurs with increased distances between pairs of trees was analysed by assessing autocorrelation within increasing distance classes. These had an annulus increase of five metres.

Pairs of 'neighbour ' trees were given a binary score of 1, if 'joined', by the pair definition, or 0, if not 'joined' within each distance class. Thus all pairs of genotypes were assessed for the number of times they co-occurred within a distance class. This number was compared to the number of joins expected and the variance for each type of join, if tree genotypes were randomly distributed (assuming sampling without replacement) (Sokal and Oden 1978a). If there were significant excesses of 'joins' between genotype pairs, those pairs of genotypes would be said to be positively autocorrelated. A deficiency of joins would indicate negative autocorrelation (Epperson and Clegg 1986).

In the case of nominal data such as these, the significance is assessed by the estimation of the Standard Normal Deviate (SND), which is compared to a T distribution (Sokal and Oden 1978a). Corrections for small sample sizes and small degrees of freedom are given by Sokal and Oden (1978a). Generally SND = (observed - expected)/ $\sqrt{}$ variance and the degrees of freedom are given by $2(\text{expected})^2/\sqrt{}$variance (Sokal and Oden 1978a). The observed and expected join counts and the variances were calculated using a FORTRAN program (written by M. Zalucki) based on the formulae given by Sokal and Oden (1978a).

Correlograms were used to investigate the patterns of association of genotypes with increasing distance at each site. A correlogram is a plot of the standard normal deviate (SND) of each pair type at each increasing distance class or annuli (Legendre and Fortin 1989). Increments of five metres were used in this analysis. To assist with interpretation of results the SND values were also averaged across all sites for each pair type. These averaged SND values were plotted. The least significant differences (LSD) for each distance class were included on the plot. The significance of correlograms was checked using the Bonferroni method of correcting for multiple tests (Oden 1984), such that at least one value is significant by this method (Legendre and Fortin 1989) where $\chi' = \chi/\upsilon$ ($\chi = 0.05$ sig level; υ = number of tests). Since some values in all correlograms were significant at Bonferroni levels the correlograms were generally considered significant (Legendre and Fortin 1989). However, the

power and significance at each distance class will be different due to the varying number of connected pairs at each distance. Therefore correlograms are most powerful at the shortest distance classes (Epperson 1989). In the present study patterns at distances greater than twenty-five metres are unlikely to be reliable due to the edge effects of the transects (Epperson 1989).

Scatter plots mapping the location, and IDH genotype of each tree were used as an aid in interpretation of spatial autocorrelation results. Distances were proportional to actual distances and the size of the symbol, which represented IDH genotype, was proportional to the actual diameters.

Other

A chi-square analysis was used to investigate whether there was a relationship between IDH genotype and gender type using results pooled across all sites. The number of IDH heterozygotes was pooled across all sites and grouped into diameter classes. The percentage of IDH heterozygotes out of the total number in each diameter class was plotted.

RESULTS

Interpretation of Isozyme Banding Patterns

Bands were scored according to their relative distance from the origin. Samples from several sites were run together to ensure direct comparison between sites. If a rare allele was scored on the gel the populations were checked for the presence of the allele in other samples in the population. If there was no other occurrence the sample was rerun to check the results. Comparison of the full data set was undertaken enabling the interpretation of observed banding patterns into loci and alleles where observed patterns fitted expectations. Adult shoot material was used for isozyme analysis, therefore the allelic designations made could not be tested on progeny. Interpretations were compared with available literature for consistency Gottlieb 1981 & 1982, Neale *et al.* 1984, Richardson *et al.* 1986). The results using freeze dried material gave results consistent with results achieved using fresh material.

Each zone of activity for IDH, 6-PG, G6PDH and AAT (nb. three zones) consistently behaved as alleles at single loci (Gottlieb 1982). The PER zymogram, however, could not be satisfactorily interpreted in terms of alleles or loci. The banding patterns at ten locations however were consistent. Therefore bands were treated as phenotypic characters and the presence or absence of these ten bands were scored for further separate analysis.

Plants usually have two isozymes for each member of the glycolytic pathway such as IDH, 6-PG and G6PDH, one in the cytoplasm and one in the chloroplast (Gottlieb 1982). Only one zone of activity (isozyme) was observed here. It is not unusual to find only one form of an enzyme, and this usually indicates that different extraction conditions are required for each to be resolved (Gottlieb 1982).

IDH

The zymograms consisted of two alternative sharp bands or a fuzzy thick band which fused both of the two closely spaced bands (Figure 3.2). The sharp bands were classed as homozygotes of two alternate alleles and the third band was classed as a heterozygote. IDH is reported to be dimeric (composed of two sub-units) (Neale *et al.* 1984). Consequently triple banded heterozygotes are expected.

6-PG and G6PDH

These enzymes were less clearly stained, however variant bands with very small differences in migration were clearly observed (Figure 3.2). There were some samples however, for which there was insufficient activity for the bands to be read (Appendix 3). There was only one region of activity (locus) observed in either enzyme (see above). Three alleles were identified in both enzymes. Alleles A (fast) and C (slow) were uncommon in both cases. These enzymes are dimers and the close proximity of bands did not allow clear definition of the expected triple-banded heterozygotes (Neale *et al.* 1984). Thick bands encompassing two locations were observed and scored as heterozygotes, but these were uncommon. Heterozygotes consisting of the two uncommon alleles were not observed. The enzymes were able to be scored in the same slice as their migration distances were very different and their substrates did not interfere with each other. The variation observed in each enzyme was independent of the other confirming the validity of this method.

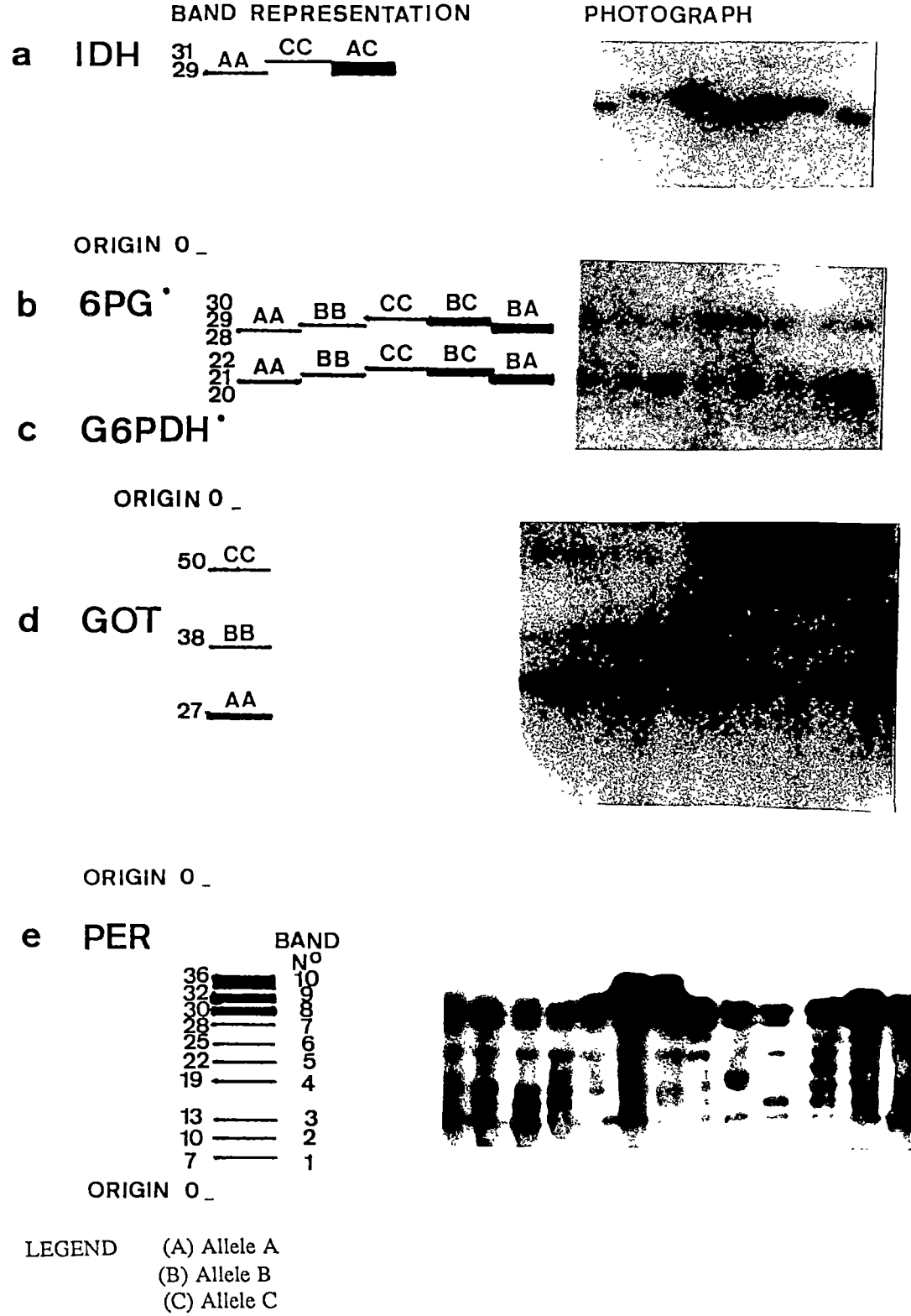
AAT

AAT's are specified by three unlinked gene loci (Gottlieb 1982). AAT-1 is the most anodal and AAT-3 the least anodally migrating. The isozymes are dimeric and interlocus heterodimers have not been observed (Gottlieb 1981). In the present study three regions (loci) of apparently homozygous monomorphic bands were observed (Figure 3.2).

PER

PER oxidise a large number of hydrogen donors including phenolic substances. Most higher plants have a large number of these enzymes (Gottlieb 1981). Their electrophoretic patterns are often complex, e.g. single alleles may sometimes specify

Figure 3.2 Examples of electrophoretic zymograms and allelic interpretations or band allocations are given for each enzyme analysed in Huon pine. Examples of the range of allelic combinations observed is illustrated and a representative example (a photograph) is given, showing variation for each enzyme). The average distance from the origin (mm) of each band is shown. Note 6PG and G6PDH were stained on the same slice but each enzyme varied independently



LEGEND (A) Allele A
(B) Allele B
(C) Allele C
Peroxidase band numbers 1-10

* These two enzymes were able to be stained simultaneously on a single slice.

enzymes in several mobility regions. Their activation is also complex due to their presence in different tissues and developmental variability (Gottlieb 1982). Allelic designations were not made here. However, ten bands were consistently scored (Figure 3.2) and treated as phenotypic charecters. The results were treated with caution, as with all phenotypic characters there is a chance of environmental effects influencing the results.

Genetic Variation

Overall very little variation in isozymes was found both within and among Huon pine sites. Most of the variation found within and among sites occurred in the PER and IDH isozymes. The two IDH alleles were fairly evenly distributed among trees (Table 3.6). There was no variation found in any of the three AAT loci scored. Both 6-PG and G-6-PDH were dominated by a single allelic form (B), however in both cases two allelic variants (A and C) were found in low frequencies (usually less than 10%) in most of the sites. One allelic variant of G6PDH (A), was present in all but 5 sites, however three of these five sites had only very small sample sizes (Table 3.6). Peroxidase bands were variable between and within sites. However, some bands were present in nearly all trees, while others alternated and some, such as number 6, were less common (Table 3.6).

There was significant variation in allelic and band frequencies among sites (Table 3.7). Five sites were dominated by IDH allele C (Lake Vera, Sir John Fall, Gordon River, Birchs Inlet, Franklin Upper) and were all located within one catchment. Those with higher proportions of allele A were more geographically widespread and isolated (Teepookana, Newall, Mt. Read, Spero). The proportions of the uncommon 6PG and G6PDH (A) and (C) alleles differed significantly among many sites (Table 3.7). Three sites had greater proportions of 6PG (A) (Farmhouse, Denison, Newall) and four had greater proportions of 6PG (C) (Irenabys, Denison, Riveaux, Spero), whilst seven sites had greater frequencies of G6PDH(A) (Table 3.7) and four sites had greater proportions of G6PDH (C) (Yellow, Spero, Riveaux, Pieman). The Spero River and Newall Creek sites overall had the greatest number of allozymes in deviating proportions (Table 3.7). Almost no genetic variation was observed within the Mt. Read site (Table 3.6). Four of the individuals which account for all of the variation found in the IDH and 6PG loci were located in a small group slightly higher than the rest of the stand and had a more pronounced krumhotlz form. Thiry-seven of the remaining 42 trees had identical genotypes. Five differed by one allele at one locus.This resulted in many significant divergences in allelic and peroxidase band frequencies and making it overall the site with the most divergent enzyme

Table 3.6. The Peroxidase band frequencies and the allele frequencies for each enzyme loci at each site.

	PEROXIDASE BAND FREQUENCIES										ALLELE FREQUENCIES AT EACH ENYME LOCUS										
POPULATION	PER 1	PER 2	PER 3	PER 4	PER 5	PER 6	PER 7	PER 8	PER 9	PER 10	IDH (A)	IDH(C)	6PG(A)	6PG(B)	6PG(C)	G6PDH(A)	G6PDH(B)	G6PDH(C)	AAT-1	AAT-2	AAT-3
Franklin Upper	0.56	0.85	0.94	0.85	0.90	0.50	0.95	1.00	0.92	0.61	0.41	0.59	0.03	0.89	0.08	0.08	0.78	0.13	1.00	1.00	1.00
Franklin Lower	0.56	0.85	0.94	0.85	0.90	0.50	0.95	1.00	0.92	0.61	0.59	0 41	0.00	0.81	0.19	0.09	0.91	0.00	1.00	1.00	1.00
Great Ravine	0.56	0 85	0.94	0.85	0.90	0.50	0.95	1.00	0.92	0.61	0.66	0.34	0.05	0.95	0.00	0.11	0.70	0.20	1.00	1.00	1.00
Franklin Ireuabys	0.56	0.85	0.94	0.85	0.90	0.50	0.95	1.00	0.92	0.61	0.47	0.53	0.02	0.74	0.24	0 05	0.77	0.19	1.00	1.00	1.00
Greystone Bluff	0.70	0 80	1.00	0.90	1.00	0.80	1.00	1.00	1.00	0.80	0.46	0.55	0.05	0.96	0.00	0.02	0.84	0.14	1.00	1.00	1.00
Frenchmans Cap	0.90	0.55	0.40	0.90	0.80	0.00	0.90	0.95	0.40	0.50	0.43	0.57	0 00	1.00	0.00	0.00	1.00	0 00	1.00	1.00	1.00
Farmhouse Creek	0.25	0.85	0.60	0.70	0.70	0.40	0.85	1.00	0.75	0 25	0.37	0.63	0.16	0.79	0.05	0.26	0.58	0.16	1.00	1.00	1.00
Teepookana	0.58	0 85	0.86	0.53	0.88	0.52	0.44	0.77	0.92	0.47	0.77	0.23	0.10	0 87	0.03	0.02	0 95	0.03	1.00	1.00	1.00
Gordon mouth	0.74	0 92	0.82	0.82	0.72	0.56	0.82	1.00	0.76	0.46	0.43	0.57	0.03	0.89	0.09	0.12	0 88	0.00	1.00	1.00	1.00
Pieman River	0.61	0.90	0.79	0.67	0.80	0.34	0.87	0.98	0.61	0.43	0.53	0.48	0.05	0.86	0.09	0.03	0.81	0.17	1.00	1.00	1.00
Denison River	0.52	0.71	0.82	0.74	0.73	0.42	0.74	1.00	0.60	0.22	0.46	0.54	0.11	0.73	0.16	0.23	0.76	0.01	1.00	1.00	1.00
Riveaux Creek	0.67	0.76	0.78	0.66	0.62	0.53	0.78	0.98	0.91	0.21	0.60	0.40	0.05	0.79	0.16	0.05	0.75	0.20	1.00	1.00	1.00
Newall Creek	0.51	0.76	0.91	0.73	0.85	0.49	0.98	1.00	0.82	0.55	0.85	0.15	0.19	0.69	0.11	0.24	0.75	0.02	1.00	1.00	1.00
King River	0.60	0.88	0.92	0.88	0.64	0.84	0.84	0.68	0.96	0.28	0.48	0.52	0.12	0.85	0.04	0.08	0.83	0.10	1.00	1.00	1.00
Pine Creek	0.71	0.61	1.00	0.63	0.78	0.24	0.93	0.98	0.90	0.49	0.43	0.58	0.08	0.95	0.00	0.26	0.74	0.00	1.00	1.00	1.00
Stanley River	0.86	0.59	1.00	0.59	0.55	0.48	0.97	0.97	0.93	0.86	0.68	0.32	0.00	1.00	0.00	0.08	0.92	0.00	1.00	1.00	1.00
Ann River	0.73	0.48	0.88	0.79	0.36	0.12	0.94	1.00	0.88	0.21	0.62	0.38	0.03	0.97	0.00	0.06	0.94	0.00	1.00	1.00	1.00
Condominium Creek	0.59	0.66	0.96	0.96	0.84	0.30	0.79	1.00	0.84	0.21	0.47	0.53	0.06	0.95	0.00	0.17	0.83	0.00	1.00	1.00	1.00
Mt Read	0.64	1.00	1.00	1.00	0.78	0.07	0.91	1.00	0.98	0.07	0.96	0.04	0.00	0.98	0.02	0.11	0.89	0.00	1.00	1.00	1.00
Spero River	0.86	0.76	0.88	0.65	0.64	0.51	0.90	0.98	0.89	0.44	0.71	0.29	0.02	0.77	0.22	0.08	0.63	0.30	1.00	1.00	1.00
Tahune Reserve	0.43	0.93	0.86	0.64	0.79	0.18	0.96	0.96	0.29	0.18	0.60	0.40	0.03	0.94	0.03	0.07	0.90	0.03	1.00	1.00	1.00
Yellow Creek	0.13	1.00	1.00	1.00	0.75	0.13	1.00	1.00	0.50	0.00	0.72	0.28	0.00	1.00	0.00	0.00	0.50	0.50	1.00	1.00	1.00
Lake Vera	0.64	0.91	0.94	0.78	0.88	0.45	1.00	1.00	0.93	0.39	0.39	0.61	0.08	0.90	0.02	0.20	0.67	0.14	1.00	1.00	1.00
Huon River	0.77	0.77	1.00	0.84	0.88	0.35	0.93	1.00	0.88	0.37	0.36	0.64	0.00	1.00	0.00	0.00	0.95	0.05	1.00	1.00	1.00
Junction Creek	0.32	0.95	0.97	0.89	0.73	0.35	1.00	1.00	0.97	0.08	0.46	0.54	0.03	0.95	0.03	0.07	0.81	0.12	1.00	1.00	1.00
Old River	0.83	1.00	0.92	0.92	0.42	0.25	0.92	1.00	0.75	0.17	0.54	0.46	0.00	0.75	0.25	0.04	0.88	0.08	1.00	1.00	1.00
Gilbert Leitch Res	0 67	0.79	0.96	0.96	0.79	0.13	0.83	1.00	0.13	0.13	0.46	0.54	0.10	0.90	0.00	0.20	0.80	0.00	1.00	1.00	1.00
Sr John Falls	0.68	0.78	0.98	0.88	0.85	0.50	1.00	1.00	0.75	0.23	0.29	0.71	0.13	0.85	0.03	0.10	0.83	0.07	1.00	1.00	1.00
Gordon River	0.76	0.88	0.71	0.69	0.50	0.51	0.94	1.00	0.90	0.53	0.42	0.57	0.11	0.89	0.09	0.20	0.70	0.10	1.00	1.00	1.00
Birches Inlet	0.44	1.00	0.94	0.63	0.94	0.81	1.00	1.00	0.91	0.34	0.32	0.69	0.00	0.94	0.06	0.12	0.72	0.17	1.00	1.00	1.00
Maxwell River	0.20	1.00	1.00	0.80	1.00	0.00	1.00	1.00	0.00	0.00	0.30	0.70	0.00	1.00	0.00	0.00	1.00	0.00	1.00	1.00	1.00
Picton River	0.50	0.97	0.88	0.81	0.81	0.66	1.00	1.00	0.94	0.63	0.54	0.47	0.00	1.00	0.00	0.00	0.89	0.12	1.00	1.00	1.00

Table 3.7. Summary table recording which PER bands are in significantly greater (+) or lower (-) proportions than expected (based on the overall average frequencies) using an analysis of deviance for each site. A summary of significant deviances of allele frequencies at each enzyme locus using a contingency Chi sqare analysis is also given. The expected frequencies were calculated first from the overall allelic frequencies, and also from allelic frequencies within each catchment. P<0.05.

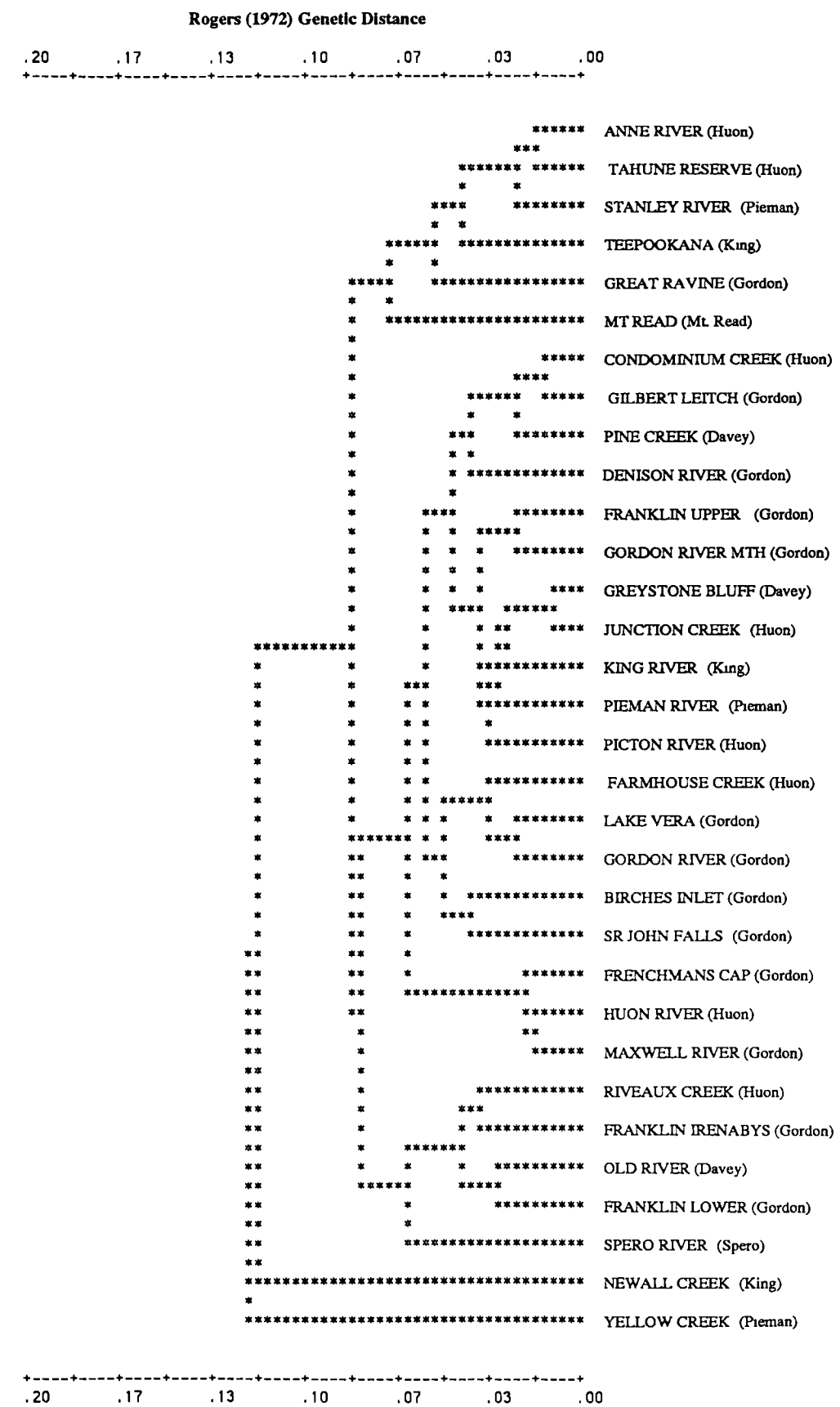
POPULATION																		WITHIN CATCHMENTS								
ENZYME	PEROXIDASE											IDH		6PG			G6PDH				IDH		6PG			G6PDH			
BAND/ALLELE	1	2	3	4	5	6	7	8	9	10	Total	A	C	A	B	C	A	B	C	Total	A	C	A	B	C	A	B	C	Total
Franklin Upper											0		+							1									0
Franklin Lower											0									0									0
Great Ravine											0									0	+	-						+	3
Franklin Irenabys					+				+	+	3					+				1					+			+	2
Greystone Bluff											0									0									0
Frenchmans Cap			-			-			-		3									0						-			1
Farmhouse Ck	-										1			+			+			2	-		+			+			3
Teepookana				-			-	-			3	+	-				-			3						-			1
Gordon mouth											0								-	1								-	1
Pieman Riv									-		1						-		+	2									0
Denison Riv									-		1			+		+	+		-	4			+		+	+		-	4
Riveaux Ck											0					+			+	2					+			+	2
Newall Ck											0	+	-	+			+		-	5		-				+			2
King Riv						+		-			2									0	-	+							2
Pine Ck											0					-	+		-	3									0
Stanley Riv										+	1								-	1								-	1
Anne Riv		-			-	-					3								-	1								-	1
Condominium Ck				+							1					-	+		-	3						+		-	2
Mt Read		+	+			-			+	+	5	+	-						-	3									0
Spero Riv	+										1	+	-			+		-	+	5					-				1
Tahune Res											0									0									0
Yellow Ck											0								+	1								+	1
Lake Vera							+				1	-	+				+			3					-				1
Huon Riv			+								1									0									0
Junction Ck	-									+	2									0									0
Old Riv											0									0					+				1
Gilbert Leitch Res									-		1									0									0
Sr John Falls											0	-	+							2									0
Gordon River			-		-						2	-	+				+			3									0
Birches Inlet		+				+					2	-	+							2									0
Maxwell Riv											0									0									0
Picton Riv											0					-	-			2			-						1

proportions (Table 3.7). When peroxidase bands were taken into account the next most divergent sites were Spero River and Teepookana (Table 3.7). There were however, some sites such as on the Gordon River, which would be expected to have greater gene flow possibilities and yet still deviated in their band proportions.The most genetically distant pairs of sites were Newall with Maxwell (0.178) and Mt. Read with Farmhouse Creek (0.172) (Table 3.8). These sites are geographically distant and isolated from other stands (Figure 3.1). Mt. Read is genetically distant from all other sites (Table 3.8). However, it is most similar to its geographically closest neighbour on the Stanley River (Table 3.8). These results reinforce the theory that the Mt. Read population has arisen vegetatively from a few founder specimens, and link it with the populations in closest geographic proximity. Genetic relationships can be used to speculate as to the relationships between other high and low altitude neighbours given the uneven nature of likely genetic exchanges (Chapter 1). For example the proportions of IDH alleles at Lake Vera reflects those of its lower altitude neighbours (Table 3.7) and this is reflected in their close genetic similarity (Table 3.8). However, the nearby high altitude Frenchmans Cap stand is quite different (Tables 3.6 & 3.8) and therefore may have a different history. The site at Pine Creek has several allozymes in proportions deviating from average (Table 3.7), whereas the nearby (high altitude) Greystone Bluff site has all allozymes present in average proportions (Table 3.7). Overall however, they are genetically relatively similar (Table 3.8). These results may indicate that the Pine Creek population is more likely to have arisen from founder trees originating in Greystone Bluff site, than visa versa.

The UPGMA tree based on genetic distance (Figure 3.3), indicated major divergences of the more geographically isolated sites, particularly, Yellow Creek (the most northerly Huon Pine site), Newall Creek, Spero, and Mt. Read. It did not however clarify other interrelations between populations (Figure 3.3). The generally close similarity between sites was demonstrated by the UPGMA tree (Figure 3.3). The UPGMA based on peroxidase band frequencies combined with allelic frequencies (Figure 3.4) segregated some geographically isolated populations from others, but the associations between sites were different from those shown in Figure 3.3, and did not correspond to any obvious grouping of sites.

Most diversity was found within sites (Table 3.9a). Genetic diversity among sites was low (Table 3.9a) and variation among sites was mostly confined to variation in allelic proportions (Table 3.6). There was little structuring of variation between catchments (Table 3.9b), the isolated catchments (Spero, King, Picman, Mt. Read), accounted for most variation observed between catchments (Table 3.10). There was

Figure 3.3 UPGMA cluster dendrogram using Rogers' (1972) Genetic Distance co-efficient, illustrating the genetic relationships among *Lagarostrobos franklinii* sites. The catchment in which each site is located is given in parenthesis.



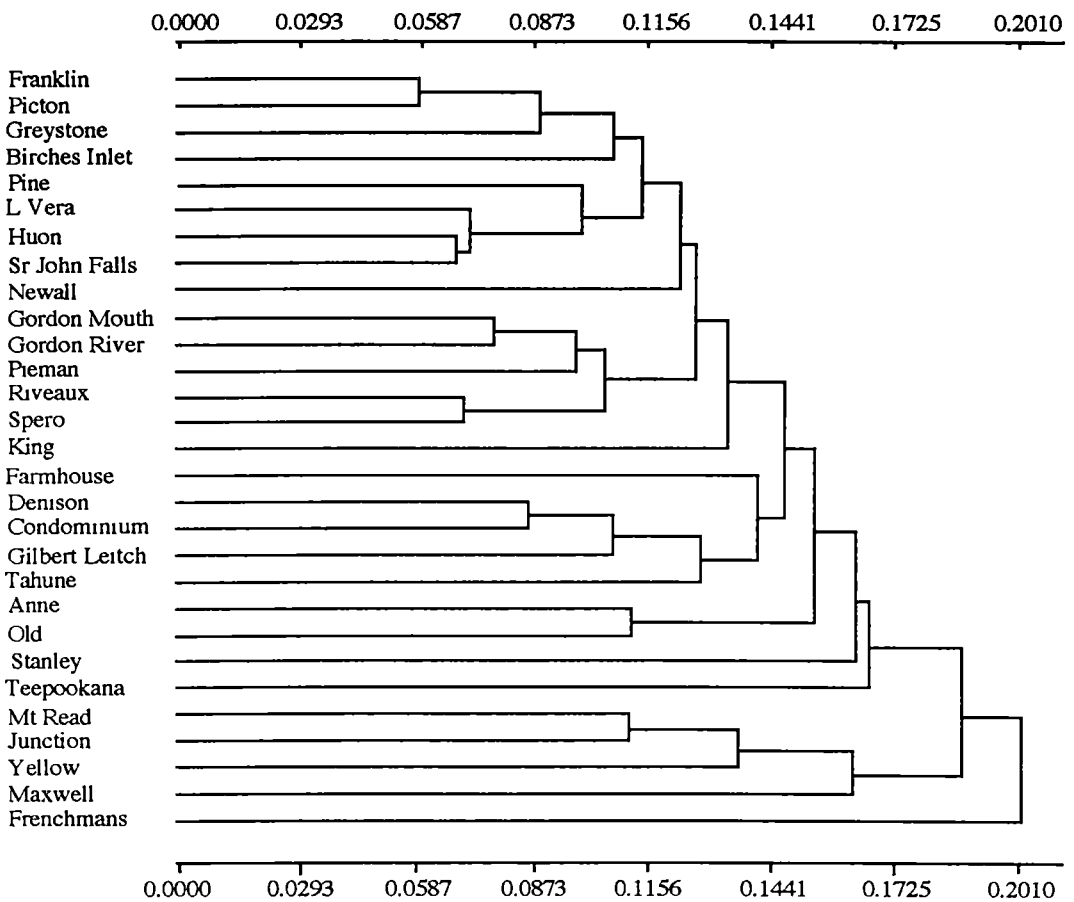
Goodness of fit statistics

Farris (1972) "f" = 8.318
Prager and Wilson (1976) "F" = 22.569
Percent standard deviation (Fitch and Margoliash,1967) = 34.825
Cophenetic correlation = .727

more structuring of variation in IDH allelic proportions (Table 3.9b). Significant deviations in IDH allelic proportions diminished when calculated by catchment (Table 3.7). 6PG and G-6-PDH diversity was independent of catchment (Tables 3.7 and 3.9b).

Generally, genetic distances among all sites were small (0.011- 0.178) (Table 3.8). The sites in the more isolated, Mt. Read, Spero, Pieman and King catchments were, however, generally more distant from sites in other catchments (Table 3.10). Sites in the Gordon, Davey and Huon catchments were not differentiated by catchment (Table 3.10). These latter three catchments all originate broadly in a common geographical area

Figure 3.4 A UPGMA dendrogram of Huon pine site relationships using a composite Euclidean distance matrix based on IDH, 6PG and G6PDH allelic frequencies and Peroxidase band frequencies.



(Figure 3.1). The most similar pairs of sites were Greystone Bluff with Junction Creek (0.011) and Condominium Creek with Gilbert Leitch (0.014). They are interspersed in the headwaters of these three river catchments (Table 3.8 and Figure 3.1). When the next most similar pairs of sites were investigated, nearly all were found to be more isolated sites in the headwaters or upper regions of the same three major river catchments (Figure 3.1 and Table 3.8).

Table 3.8. A matrix of genetic distances between Huon pine sites based on IDH, 6PG, G6PDH and AAT allozyme analysis. Above the diagonal Roger's (1972) genetic distance. Below the diagonal Wright's (1978) modified Roger's (1972) distance.

POPULATION	CODE	ANNE	COND	PINE	FARM	FCAP	FRAU	GREY	GORM	KING	VERA	READ	NEWL	PICT	PIEM	RIVE	SPER	STAN	TEEP	TAHU	DENS	YELL	JUNC	HUON	MAXL	OLDR	BIRC	GILB	FRAI	JOHN	GORD	GRAV	FRAL
ANNE RIVER	ANNE	*****	0047	0074	0121	0047	0073	0051	0056	0059	0089	0068	0109	0038	0058	0064	0101	0018	0045	0015	0091	0101	0052	0058	0069	0065	0093	0062	0095	0089	0093	0048	0039
CONDOMINIUM CREEK	COND	0077	*****	0026	0077	0045	0042	0028	0029	0032	0046	0099	0112	0043	0049	0077	0117	0060	0084	0041	0044	0124	0026	0054	0067	0069	0060	0014	0066	0057	0050	0060	0061
PINE CREEK	PINE	0116	0042	*****	0056	0057	0041	0045	0036	0047	0030	0125	0108	0067	0065	0089	0123	0086	0103	0066	0041	0134	0041	0064	0077	0089	0056	0020	0077	0057	0034	0073	0083
FARMHOUSE CREEK	FARM	0177	0113	0084	*****	0103	0059	0081	0073	0065	0032	0172	0120	0106	0081	0090	0111	0134	0134	0108	0059	0142	0075	0089	0104	0103	0058	0064	0079	0058	0033	0095	0109
FRENCHMANS CAP	FCAP	0083	0076	0111	0171	*****	0051	0037	0037	0057	0071	0110	0156	0035	0066	0098	0142	0055	0083	0052	0084	0132	0041	0020	0021	0079	0070	0055	0084	0071	0074	0092	0074
FRANKLIN UPPER	FRAU	0110	0062	0072	0094	0087	*****	0029	0025	0032	0032	0124	0128	0051	0033	0058	0099	0084	0095	0057	0056	0124	0021	0049	0065	0062	0032	0042	0044	0043	0032	0067	0069
GREYSTONE BLUFF	GREY	0085	0059	0087	0124	0064	0042	*****	0038	0028	0049	0110	0135	0026	0032	0065	0109	0065	0082	0044	0065	0111	0011	0041	0058	0061	0051	0037	0052	0054	0055	0057	0071
GORDON RIVER MTH	GORM	0089	0042	0065	0119	0065	0049	0060	*****	0035	0048	0104	0121	0053	0044	0073	0114	0067	0083	0045	0048	0141	0033	0047	0059	0058	0054	0032	0059	0047	0041	0083	0047
KING RIVER	KING	0084	0053	0075	0104	0086	0046	0045	0051	*****	0047	0114	0107	0042	0028	0053	0097	0073	0070	0046	0044	0125	0023	0062	0078	0048	0061	0030	0045	0037	0039	0064	0056
LAKE VERA	VERA	0138	0073	0052	0051	0126	0053	0079	0082	0073	*****	0141	0124	0074	0059	0079	0107	0102	0109	0077	0057	0124	0044	0063	0078	0091	0035	0038	0069	0047	0024	0066	0093
MT READ	READ	0139	0200	0228	0274	0220	0231	0211	0218	0204	0248	*****	0083	0094	0113	0117	0122	0055	0062	0073	0139	0119	0106	0119	0131	0120	0141	0113	0147	0143	0143	0090	0091
NEWALL CREEK	NEWL	0156	0181	0192	0210	0228	0204	0201	0194	0170	0207	0125	*****	0133	0112	0094	0089	0101	0076	0106	0078	0136	0127	0164	0178	0109	0149	0103	0120	0136	0102	0095	0096
PICTON RIVER	PICT	0058	0070	0106	0154	0062	0072	0038	0075	0066	0109	0182	0192	*****	0031	0064	0107	0043	0072	0034	0083	0097	0031	0039	0057	0051	0069	0057	0069	0076	0078	0059	0060
PIEMAN RIVER	PIEM	0084	0076	0101	0121	0097	0053	0048	0072	0040	0089	0190	0167	0056	*****	0033	0077	0071	0073	0043	0060	0107	0031	0070	0088	0040	0061	0052	0039	0064	0053	0054	0050
RIVEAUX CREEK	RIVE	0106	0110	0128	0133	0140	0091	0095	0107	0076	0115	0176	0138	0096	0047	*****	0045	0077	0078	0051	0065	0098	0060	0103	0120	0043	0080	0078	0036	0090	0064	0046	0039
SPERO RIVER	SPER	0156	0166	0177	0168	0203	0150	0155	0170	0140	0161	0174	0131	0152	0111	0068	*****	0092	0087	0094	0096	0069	0103	0146	0163	0072	0113	0116	0066	0133	0096	0057	0074
STANLEY RIVER	STAN	0028	0097	0133	0193	0108	0131	0106	0113	0107	0156	0113	0147	0076	0101	0114	0153	*****	0042	0028	0102	0085	0064	0065	0076	0077	0102	0074	0106	0102	0105	0048	0049
TEEPOOKANA	TEEP	0071	0136	0170	0213	0147	0161	0138	0146	0125	0186	0094	0113	0114	0116	0112	0141	0062	*****	0047	0107	0103	0084	0090	0104	0081	0124	0084	0110	0101	0107	0066	0063
TAHUNE RESERVE	TAHU	0022	0065	0104	0159	0080	0091	0070	0075	0066	0121	0149	0151	0048	0063	0086	0140	0043	0077	*****	0078	0103	0040	0059	0073	0050	0079	0053	0080	0076	0079	0047	0029
DENISON RIVER	DENS	0129	0083	0076	0086	0137	0084	0110	0074	0072	0084	0226	0160	0128	0092	0099	0147	0146	0159	0111	*****	0153	0056	0093	0106	0058	0078	0034	0046	0069	0034	0092	0062
YELLOW CREEK	YELL	0198	0208	0217	0207	0237	0191	0182	0224	0190	0192	0209	0210	0176	0162	0146	0117	0191	0194	0188	0224	*****	0110	0136	0154	0138	0126	0133	0133	0159	0136	0064	0129
JUNCTION CREEK	JUNC	0084	0048	0073	0113	0072	0031	0019	0051	0041	0067	0208	0193	0044	0044	0088	0147	0104	0139	0067	0097	0180	*****	0045	0062	0058	0042	0032	0046	0051	0048	0055	0066
HUON RIVER	HUON	0111	0082	0107	0156	0035	0074	0061	0067	0088	0111	0248	0247	0074	0100	0146	0209	0135	0174	0103	0137	0237	0068	*****	0017	0084	0051	0064	0088	0053	0076	0097	0084
MAXWELL RIVER	MAXL	0134	0102	0123	0171	0052	0097	0090	0084	0111	0129	0272	0270	0103	0128	0173	0237	0159	0197	0128	0152	0267	0096	0030	*****	0100	0054	0077	0106	0049	0092	0113	0096
OLD RIVER	OLDR	0106	0108	0129	0149	0121	0091	0102	0084	0081	0129	0196	0161	0103	0067	0064	0118	0121	0124	0088	0086	0205	0095	0130	0149	*****	0092	0073	0033	0083	0073	0084	0031
BIRCHES INLET	BIRC	0150	0091	0083	0088	0113	0047	0076	0084	0089	0050	0271	0243	0108	0097	0131	0181	0170	0205	0134	0112	0206	0068	0088	0104	0133	*****	0062	0068	0040	0049	0071	0097
GILBERT LEITCH	GILB	0092	0022	0031	0097	0092	0064	0069	0047	0050	0063	0209	0177	0086	0081	0111	0166	0112	0144	0079	0069	0212	0058	0094	0112	0110	0090	*****	0066	0050	0040	0070	0067
FRANKLIN IRENABYS	FRAI	0136	0114	0123	0115	0136	0072	0097	0092	0079	0102	0230	0184	0113	0063	0062	0112	0152	0160	0115	0082	0188	0089	0131	0151	0053	0103	0112	*****	0077	0053	0080	0060
SR JOHN FALLS	JOHN	0147	0087	0084	0098	0100	0063	0082	0070	0077	0072	0277	0240	0116	0105	0142	0203	0172	0199	0132	0099	0243	0081	0078	0083	0131	0063	0080	0112	*****	0050	0097	0087
GORDON RIVER	GORD	0133	0073	0057	0050	0128	0054	0086	0072	0061	0038	0239	0185	0114	0080	0097	0146	0151	0173	0114	0049	0200	0073	0119	0137	0103	0071	0059	0079	0074	*****	0077	0077
GREAT RAVINE	GRAV	0093	0101	0121	0144	0146	0111	0099	0126	0095	0118	0146	0135	0089	0077	0073	0092	0090	0105	0082	0131	0114	0093	0155	0184	0123	0143	0109	0124	0163	0118	*****	0073
FRANKLIN LOWER	FRAL	0073	0090	0118	0160	0109	0100	0102	0078	0081	0134	0163	0140	0092	0074	0077	0129	0085	0095	0059	0089	0210	0094	0128	0147	0045	0145	0096	0090	0140	0111	0111	*****

Table 3.9. (A) Summary of Wright's (1965) F statistics averaged over all Huon pine sites. The statistics partition the genetic diversity into its components within and among sites. (B) Hierarchical F statistics (Wright 1978) analyse the partitioning of genetic diversity among sites to identify whether genetic diversity is partitioned among catchments.

(A)

Enzyme	F(IS) (ind/site)	F(IT) (ind/tot)	F(ST) (sites/tot)
IDH	0.522	0.569	0.099
6PG	0.865	0.876	0.082
G6PDH	0.872	0.884	0.097
MEAN	0.702	0.73	0.095

(B)

Enzyme	Among X	Within Y	Variance component	Diversity F(XY)
IDH	Site	Catchment	0.01675	0.035
	Site	Total	0.04109	0.082
	Catchment	Total	0.02435	0.049
6PG	Site	Catchment	0.01264	0.062
	Site	Total	0.01336	0.065
	Catchment	Total	0.00072	0.004
G6PDH	Site	Catchment	0.02834	0.086
	Site	Total	0.02708	0.083
	Catchment	Total	0.00125	-0.004
MEAN	Site	Catchment	0.05772	0.057
	Site	Total	0.0815	0.0079
	Catchment	Total	0.02381	0.023

The A or C alleles of 6PG and G6PDH were found to be positively correlated with each other ($p \leq 0.0025$, Table 3.11) However, they were found to be associated with particular climatic conditions in opposite ways (Table 3.11). G6PDH and 6PG A forms were positively ($p = 0.0235$ & 0.0055), and the G6PDH C form negatively, correlated ($p = 0.0378$) with the climate factor 3. There were also independent associations with other climatic conditions. For example, the negative correlation of G6PDH(C) with climatic factor 1 ($p = 0.022$), and the positive correlation of 6PG(A) with climatic factor 2 ($p = 0.0239$). These results suggest the possibility of some selection. The C forms of both enzymes were correlated with location, such that they were more frequent at lower altitudes to the west, because there is a negative correlation with both easting and altitude ($p < 0.028$. Table 3.11). However, neither IDH allelic frequencies nor average genetic distances were correlated with geography or climate (Table 3.11 and 3.12). Allelic frequencies or genetic distances were not correlated to any other environmental or ecological variables used for comparison (Tables 3.11 and 3.12).

Table 3.10. A matrix of genetic distances (Rogers 1972) between Huon pine sites, averaged by catchment. The range of genetic distances between sites within each catchment is given in parenthesis. (*****) no comparisons.

CATCHMENT	No.of sites.	GORDON	HUON	KING	DAVEY	PIEMAN	SPERO	MT READ
GORDON	13	0.062 (.021-.113)						
HUON	8	0.062 (.014-.120)	0.061 (.015-.121)					
KING	3	0.088 (.030-.178)	0.083 (.023-.164)	0.084 (.070-.107)				
DAVEY	3	0.058 (.020-.100)	0.056 (.011-.103)	0.082 (.028-.135)	0.065 (.045-.089)			
PIEMAN	3	0.09 (.033-.159)	0.075 (.018-.142)	0.088 (.028-.136)	0.083 (.032-.138)	0.087 (.071-.107)		
SPERO	1	0.106 (.057-.163)	0.103 (.045-.146)	0.091 (.087-.097)	0.101 (.072-.123)	0.079 (.069-.092)	***** (*****-*****)	
MT READ	1	0.124 (.090-.147)	0.106 (.068-.172)	0.086 (.062-.114)	0.118 (.110-.125)	0.095 (.055-.119)	0.122 (.122-.122)	***** (*****-*****)

Table 3.11. Summary of significant correlations (p<0.05) between reproductive, ecological, environmental, geographic and genetic variables as assessed by a Spearmans Rank correlation analysis. (+) and (-) denote the sign of the correlations. The probability of each correlation is also given.

VARIABLES	Easting	Northing	Altitude	Climate 1	Climate 2	Climate 3	Density	Seedlg Density	Females	Reprod	IDHA	6PGA	6PGC	G6PDH A	G6PDH C	Heterozy	IDH Fixation	No Sig Bands
Easting	***	(-) 0.0001	(+) 0.0053		(-) 0.0001	(+) 0.005	(+) 0.028	(+) 0.0001					(-) 0 0268		(-) 0.0112			
Northing		***			(+) 0.0001	(-) 0.0041	(-) 0.0487	(-) 0.0002										
Altitude			***	(+) 0.0001	(-) 0.0051					(-) 0.0089			(-) 0.0012		(-) 0.0109			
Climate Factor1				***											(-) 0 022			
Climate Factor2					***		(-) 0.0332	(-) 0.0005				(+) 0.0239						
Climate Factor3						***		(-) 0.0545				(+) 0.0055		(+) 0.0235	(-) 0.0378			
Density							***	(+) 0.0312										
Seedling Density								***		(-) 0.0345								(-) 0.0005
Proportion Females									***									
Proportion Reproductive										***								
Frequency IDH A											***							
Frequency 6PG A												***		(+) 0.0016	(+) 0 0165			
Frequency 6PG C													***		(+) 0.0025			
Frequency G6PDH A														***		(+) 0.0019	(-) 0 0033	
Frequency G6PDH C															***			
Heterozygosity																***	(-) 0.0001	
IDH fixation																	***	

Table 3.12. Summary of Mantels G values for tests comparing pairs of distance matrices between Huon pine sites. * P< 0.05

Variables	Reprodtn	Female	Density	Sructure	Disturb	Geography	Species	Climate
Reproduction								
Females	0.479							
Density	-0.66	-0.1						
Structure	-1.48	-0.01	-0.37					
Disturbance	*3.78	-0.36	1.275	*2.73				
Geography	0.2	-0.46	*2.305	-1.49	1.66			
Species	0.58	*2.25	0.81	1.25	1.26	0.46		
Climate	-1.41	1.25	0.66	0.48	*3.46	0.23	*3.04	
Genetics	0.177	*3.70	1.19	0.14	-0.01	1.39	1.12	1.43

Since estimates of gender expression were available (Chapter 2) the question arose as to whether there were any correlations between gender, reproduction and genotype. No correlations were found between allele frequencies, genetic distance and reproductive activity (Tables 3.11 and 3.12). Since IDH alternative alleles were present in roughly equal proportions within populations, IDH genotypes and gender type were compared directly to investigate any relationships, however no significant relationships were found, and the proportion of females was not correlated to allelic frequencies of any loci (Table 3.11). However, total genetic distance was positively correlated to the proportions of females present (Table 3.12). As seen earlier (Chapter 2) gender expression is correlated with species composition (Table 3.12), but not the other ecological variables measured (Tables 3.11 and 3.12). This result therefore suggests some ecological factor which may affect gender proportions, has affected genotypic proportions. It also possibly suggests that environmental determination of sex ratios in Huon pine populations may have a genetic basis.

Breeding System

Although Huon pine is predominantly an obligate outcrosser, being mostly dioecious (Chapter 2), it is characterised by conspicuous vegetative reproduction and unequal reproductive contributions between trees (Chapter 2). Isozyme analysis was also used to investigate the effective balance of this breeding system. It was not surprising to find that the sites showed significant negative deviations from the genotypic proportions expected under Hardy-Weinberg conditions for all the three enzyme systems (Table 3.13). The least deviation was seen in the most variable enzyme IDH, with 8 out of 32 sites conforming to Hardy-Weinberg expectations. However, one of these sites was Mt. Read, it has, as we have seen almost no within site variation (Table 3.6), and hence given the sample size, almost no heterozygotes are expected or were observed (Table 3.14).

Table 3.13. Deviation of the frequencies of heterozygotes from that expected under Hardy - Weinberg conditions ($p< 0.05$) and Wrights (1965) Fixation indexes for each enzyme in Huon pine sites. (1 = completely fixed, 0 = Hardy - Weinberg equilibrium, -1 = complete heterozygote excess). * No enzyme variation.

	Fixation Index F			Deviation from H-W		
POPULATION	IDH	6PG	G6PDH	IDH	6PG	G6PDH
Anne River	0.163	1.000	1.000	ns	sig	sig
Condominium Creek	0.344	1.000	0.682	sig	sig	sig
Pine Creek	0.334	1.000	0.935	sig	sig	sig
Farmhouse Creek	0.548	1.000	0.816	sig	sig	sig
Frenchmans Cap	0.417	*	*	ns	*	*
Franklin Upper	0.414	1.000	0.908	sig	sig	sig
Greystone Bluff	1.000	1.000	0.834	sig	sig	sig
Gordon Mouth	0.592	0.793	0.933	sig	sig	sig
King River	0.461	1.000	0.872	sig	sig	sig
Lake Vera	0.585	1.000	1.000	sig	sig	sig
Mt Read	-0.050	1.000	1.000	ns	sig	sig
Newall Creek	0.516	0.888	0.864	sig	sig	sig
Picton River	0.949	*	0.874	sig	*	sig
Pieman River	0.599	0.898	1.000	sig	sig	sig
Riveaux Creek	0.063	0.958	0.851	ns	sig	sig
Spero River	0.728	0.898	0.927	sig	sig	sig
Stanley River	0.651	*	0.784	sig	*	sig
Teepookana	0.803	1.000	0.654	sig	sig	sig
Tahune Reserve	0.665	1.000	1.000	sig	sig	sig
Denison River	0.298	0.860	0.870	sig	sig	sig
Yellow Creek	0.723	*	0.778	sig	*	sig
Junction Creek	0.674	1.000	0.833	sig	sig	sig
Huon River	0.689	*	1.000	sig	*	sig
Maxwell River	-0.429	*	*	ns	*	*
Old River	0.497	1.000	0.631	ns	sig	sig
Birches Inlet	0.307	1.000	0.775	ns	sig	sig
Gilbert Leitch	0.597	-0.111	1.000	sig	ns	sig
Franklin Irenabys	0.749	0.812	0.917	sig	sig	sig
Sr John Falls	0.647	0.809	0.836	sig	sig	sig
Gordon River	0.796	0.561	0.893	sig	sig	sig
Great Ravine	0.761	-0.057	0.923	sig	ns	sig
Franklin Lower	0.093	1.000	0.632	ns	sig	sig

Even though the expected average heterozygosity was low (17% overall and ranged from 10% to 20% within sites), sites were generally deficient in heterozygotes (Table 3.14). The observed average heterozygosity was only 4% and ranged from 1% to 8% within sites (Table 3.14). The sites with the lowest average heterozygosities were Greystone Bluff, Mt. Read and Picton and those with the highest were Maxwell, Franklin Lower and Riveaux (Table 3.14). Since the variant forms of both 6PG and G-6-PDH were uncommon it is unsurprising that the heterozygote genotypic proportions were low and therefor most sites were almost completely fixed for the common genotype (Table 3.13). The IDH fixation index

index (Wright 1965) was used as a measure of inbreeding as its two alleles were well represented in most populations. A score of +1 indicates total inbreeding, complete allelic fixation, or complete heterozygote deficiency, 0, a population in Hardy-Weinberg equilibrium, and -1 a complete heterozygote excess. Populations with a fixation index significantly greater than 0 are said to have significant fixation of allelic proportions.

Table 3.14. Summary of genetic variability measures averaged over all loci in Huon pine sites.

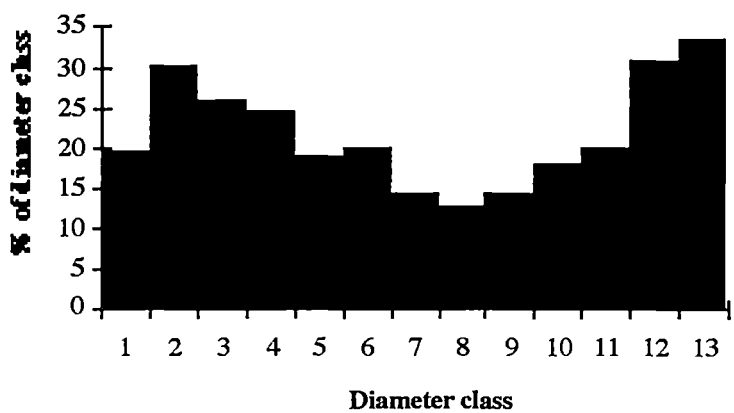
POPULATION	Mean sample size per Locus N	Mean no. of alleles per locus A	Percentage of loci polymorphic* P	Mean Heterozygosity	
				Direct-count Ho	HdyWbg expected** He
ANNE RIVER	33	1.5	50	0.066	0.109
CONDOMINIUM CREEK	55	1.5	50	0.07	0.149
PINE CREEK	40	1.5	50	0.054	0.171
FARMHOUSE CREEK	19	1.8	50	0.053	0.237
FRENCHMANS CAP	21	1.2	16.7	0.048	0.084
FRANKLIN UPPER	56.3	1.8	50	0.053	0.175
GREYSTONE BLUFF	22	1.7	50	0.008	0.146
GORDON RIVER MOUTH	70	1.7	50	0.043	0.153
KING RIVER	26	1.8	50	0.051	0.182
LAKE VERA	63.2	1.8	50	0.033	0.195
MT READ	46	1.5	50	0.014	0.054
NEWALL CREEK	57	1.8	50	0.038	0.187
PICTON RIVER	39	1.3	33.3	0.009	0.119
PIEMAN RIVER	120	1.8	50	0.038	0.178
RIVEAUX CREEK	69	1.8	50	0.087	0.204
SPERO RIVER	107	1.8	50	0.031	0.216
STANLEY RIVER	33	1.3	33.3	0.03	0.097
TEEPOOKANA	69.5	1.8	50	0.017	0.114
TAHUNE RESERVE	31	1.8	50	0 027	0 133
DENISON RIVER	83	1.8	50	0 076	0 218
YELLOW CREEK	9	1.3	33.3	0 037	0 159
JUNCTION CREEK	37	1.8	50	0 036	0 156
HUON RIVER	21	1.3	33.3	0 024	0.094
MAXWELL RIVER	5	1.2	16 7	0.1	0.078
OLD RIVER	12	1.7	50	0 056	0.191
BIRCHES INLET	27 8	1.7	50	0 067	0.168
GILBERT LEITCH	21.7	1.5	50	0 067	0.172
FRANKLIN IRENAB	31 2	1 8	50	0 038	0.215
SR JOHN FALLS	37 5	1 8	50	0 041	0 165
GORDON RIVER	94 5	1 8	50	0 049	0.214
GREAT RAVINE F	28	1 7	50	0.042	0 172
FRANKLIN LOWER	16	1 5	50	0.083	0.165
WEIGHTED MEAN	43.8	1.6	46	0.044	0 169

*** A locus is considered polymorphic if more than one allele was detected**
**** Unbiased estimate (see Nei, 1978)**

Most Huon pine sites studied were inbred by this measure but there was considerable variation in degree (Table 3.13), and some sites, such as Anne, Franklin lower, Riveaux and Maxwell (NB small sample size) were not inbred (Table 3.13). It is interesting that two sites from along the big river system fringes, (Picton and Gordon), were amongst the most inbred stands rather than the least as may have been anticipated (Figure 3.1 and Table 3.13). The degree of inbreeding

and the proportions of heterozygotes were not correlated to current levels of reproductive activity, nor could any other explanatory patterns be found (Table 3.11). Overall, heterozygotes were fairly evenly represented through all tree sizes, there were no significant differences in the proportions of heterozygotes between the size classes examined. However there were slightly greater proportions of heterozygotes found at the two extremes of the size range (Figure 3.5). Examination of heterozygotes in individual populations revealed that their size distribution reflected the overall size distribution of the population. Population maps showing genotype and sex indicated that heterozygotes were scattered within populations and there was no evidence of the formation of clonally derived clumps of heterozygotes.

Figure 3.5 A plot of the percentage of sampled trees in each diameter class which have heterozygous IDH genotypes. The data is pooled across all Huon pine sites. Diameter size classes; 1 = 0 - 2; 2 = 3 - 5; 3 = 6 -10; 4 = 11 - 15; 5 = 16 - 20; 6 = 21 - 25; 7 = 26 - 30; 8 = 31 - 40; 9 = 41 - 50; 10 = 51 - 70; 11 = 71 - 90; 12 = 91 - 110; 13 = 111 +.



Spatial genetic structure within sites

Two major patterns in site genotypic structure were revealed by the spatial autocorrelation analysis. At short distances (10m) genotypes of the same kind were strongly positively associated with one another, whereas trees of different genotypes were unlikely to be neighbours (Table 3.15). These patterns were consistently found in all the Huon pine sites (Table 3.15). Those sites non-significant for a pair type usually had low frequencies of that combination of genotypes. When these associations were investigated with increasing distance, again there were two distinct patterns observed (Figure 3.6 and Table 3.15). The positive association between individuals with the same IDH genotype, as measured by their SND, decreased with distance, with the SND becoming negative (Figure 3.6). In contrast the trees with unlike genotypes remained separated from one another at all distances (Table 3.15). The point at which correlograms cross the origin (SND=O) has been equated with patch size (Epperson and Clegg 1986). If this is so then for Huon pine there is a remarkable consistency of patch size of about 15 metres radius among sites

(Figure 3.6). These results coincide almost exactly with the patch size of like gender associations found in the same Huon pine stands (Chapter 2).

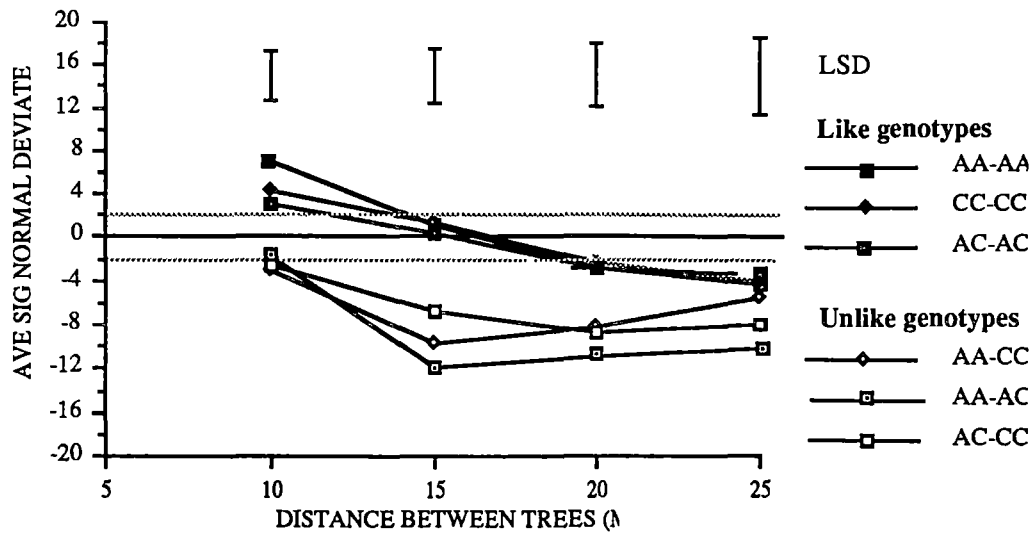
There were some sites with larger patch sizes for trees with like genotypes, Greystone Bluff and Teepookana were the most marked examples. These two sites also had almost no observed heterozygotes (Table 3.15) and very high IDH fixation indices (Table 3.14). Both sites were part of large non-linear shaped populations away from major rivers, with higher proportions of large, widely spaced trees.

Table 3.15 Summary of spatial autocorrelation results over all Huon pine sites studied. The results of each IDH genotype tree pair combination are pooled over all study sites and given as percentages of the total number of each type of genotypic tree pair combination. The results are given as like genotypic pair combinations and unlike pair combinations. Trees within a 10 metre radius were paired, as were trees within a 10 to 15 metre radius, and trees within a 15 to 20 metre radius. P<0.05.

Pairs	% sig +ve	% non sig	% sig -ve
10 metres			
Like			
AA-AA	70.6	23.5	5.9
CC-CC	70.6	29.4	0.0
AC-AC	41.2	41.2	5.9
Unlike			
AA-CC	5.9	17.6	76.5
AA-AC	0.0	47.1	46.7
AC-CC	0.0	47.0	46.7
15 metres			
Like			
AA-AA	35.7	42.9	21.4
CC-CC	40.0	60.0	0.0
AC-AC	13.3	86.7	0.0
Unlike			
AA-CC	0.0	0.0	100.0
AA-AC	0.0	0.0	100.0
AC-CC	0.0	13.3	86.7
20 metres			
Like			
AA-AA	17.6	11.8	76.5
CC-CC	5.9	35.3	58.8
AC-AC	0.0	31.3	68.8
Unlike			
AA-CC	0.0	11.7	88.2
AA-AC	0.0	6.7	93.3
AC-CC	0.0	0.0	100.0

The trend to large negative SND values at increasing distances for trees with like genotypes is probably largely a statistical artifact produced by too few neighbouring trees at larger distances due to the long narrow nature of most stands (reflecting transect shape). This pattern has been noted by other authors (Sokal and Oden 1978a).

Figure 3.6 Correllogram of IDH genotype autocorrelation associations The significant normal deviate scores (SND) for each pair category were averaged over all sites. The correllogram illustrates the change in the mean significant normal deviate for each genotype pair with increasing distances between pairs of trees. The dotted lines indicate the p<0.05 significance level for SND values. The Least significant difference (LSD) between the pooled SND values is indicated by the error bars at each distance class.



DISCUSSION

Most variation was found within rather than among Huon pine sites, and sites differed mostly in allelic frequencies rather than allelic composition. The diversity among Huon pine sites (Fst ~ Gst = 0.095) (Nei 1977) was low compared to other plants overall (Gst = 0.224) (Hamrick and Godt 1989). However, the level of differentiation among Huon pine stands is comparable to other wind pollinated (0.100) or late successional species (0.101) and similar but higher than other gymnosperms (0.068) or long-lived woody species (0.076) (Hamrick and Godt 1989).

The most genetically distinctive sites were also the most isolated, such as Teepookana, Spero and Mt. Read (Table 3.7, Figure 3.3). This result may be expected, and suggests that there is less gene flow to more isolated sites. There was some evidence of differentiation in stands on the more isolated river catchments (Table 3.7), but there was no pattern of differentiation of sites between the three major catchments of the Gordon, Huon and Davey rivers (Table 3.10) although there was variation between individual sites (Table 3.6). These three river systems however, originate in a similar region (Figure 3.1), and the most similar pairs of sites overall were interspersed within the headwaters of these three catchments (Table

3.8). Such similarity could reflect pollen flow, similar habitats, or may indicate a more relictual link between these sites.

There appears to be a geographic trend in the presence of the uncommon (C) alleles of both 6PG and G6PDH. (Table 3.11) Significant correlations suggest that these alleles occur more frequently in the more westerly lower altitude sites. Their occurrence mostly in lower altitude sites may reflect their low frequency (mostly less than 10% when present) and hence lower probability of being represented in infrequent uphill migration events, but may also indicate selection for or against this form. The alternate directions of correlations of G6PDH allelic forms A and C with particular climatic conditions (factor 3) (Table 3.11) may also suggest some differential selection for these forms at different sites. The frequencies of both of the uncommon forms of 6PG and G6PDH (A and C) are correlated which may suggest linkage. However, Ennos (1989) found that significant correlations in allelic state may be found in highly inbred populations whether or not loci are linked, and also suggested that allelic differences found at marker loci are therefore likely to represent differences at many loci.

The IDH B allele was found in high frequencies in many of the sites in the Franklin-Gordon river area, including at Lake Vera (Table 3.7), which is located close by, but at a much higher altitude. With the exception of Lake Vera, these are sites which would be expected to receive perhaps the greatest gene flow from other sites, as this is a very large catchment in the heart of the Huon pine distribution (Peterson 1990). Therefore the higher proportion of the B form is unlikely to be due to drift. That the B form dominates here and the alternative A form dominates in some of the isolated sites towards the edge of its distribution (Table 3.7), may reflect a selectional difference, the isolated sites may also have differentiated due to chance drift but the results would be expected to be random. There is no evidence for IDH allelic correlation with climate, altitude or geographic location (Table 3.11).

Overall there was no correlation between genetic distance and geographic distance (Table 3.12) and there was little found to explain the patterns of genetic variation that were observed among Huon pine stands. Genetic distance did not parallel climatic difference (Table 3.12), although as shown above there were some individual allelic associations, and there was no association with species composition, another ecological indicator (Table 3.12). Genetic differentiation and allelic proportions did not appear to be related to stand structure, density or amount of disturbance (Tables 3.11 & 3.12).

Genetic distance was correlated to the proportion of females present in stands (Table 3.12), but individual loci were not correlated to the proportion of females (Table 3.11), nor any IDH genotype to a particular gender. This result however does suggest that both genetics and gender (Chapter 2), are responding to selection pressures due to some aspect of the environment. As suggested in the previous chapter there is indirect evidence for a correlation between the proportion of females and soil fertility. Unfortunately soil fertility was not determined in this study but it is a likely factor which could also direct allelic selection at individual sites. The apparently mostly random pattern of variation in allelic frequencies between sites may be due to selection for individual site conditions, and/or may also be the result of the chance allelic proportions in the small founder or bottleneck populations which gave rise to many of the current stands. Since there is a low rate of seed germination and establishment, there may be strong microhabitat selection, or there may also be a large chance component to the survival of germinants.
In the absence of selection populations in closer geographic proximity would be expected to be more similar than geographically distant ones. While correlations between genetic distance and geographic distance have been found in some trees (Yeh and O'Malley 1980, Van Treuren *et al.* 1991, Gurries and Ledig 1978, Moran and Hopper 1983), others have found no correlation (Barratt and Husband 1989, Schwaegerle and Schaal 1979, Prober *et al.* 1990 and Xie *et al.* 1992). The results of Xie *et al.*'s (1992) study of *Thuja orientalis* were similar to this study as they found some geographical trends at individual loci, but overall no correlation between genetic and geographical distances.

Van Treuren *et al.* (1991) found that differentiation was greater between small populations than larger ones. Xie *et al.* (1992) suggested that the tendency of *T. orientalis* to occur as small populations contributed to its localised differentiation since small populations are more likely to be affected by inbreeding and drift (Barratt and Husband 1989). Loveless and Hamrick (1984) suggested that there is likely to be increased divergence between populations which occur in linear arrays, such as along rivers, due to unidirectional gene flow, and that there is likely to be increased subdivision along such linear arrays. Huon pine occurs in small stands within larger highly subdivided often linear populations along water courses, and therefore it is expected that there would be unidirectional gene flow. These features are likely to have contributed to the localised differentiation of Huon pine stands.

It has been suggested that relatively low levels of genetic diversity among populations of long-lived late successional gymnosperms, of which Huon pine is typical, are probably largely the result of their long life cycle and open breeding

systems (Gurries and Ledig 1981). Sufficient gene flow to reduce differentiation is more likely to occur when a species' life span is long. Many long-lived species are also said to be outcrossed (Loveless and Hamrick 1984, Moran and Bell 1983). Species which are inbred tend to generally have higher among population differentiation (Brown 1979, Loveless and Hamrick 1984).

Here lies the paradox, as Huon pine is highly inbred, even though it is largely dioecious and therefore mostly an obligate outcrosser. For example its average heterozygosity (0.044) is only one quarter of the level expected (0.17) and is less than half an estimated average for plants generally (0.11) (Levin 1975). Eucalypts (Prober *et al.* 1990), some gymnosperms (Yeh 1981, Xie *et al.* 1992) and long lived *Macrozamia* (Bryne and James 1991) all have higher levels of heterozygosity. The rare *Banksia cuneata* has similar low levels of heterozygosity, but they do not differ from those expected (Coates and Sokolowski 1992). The heterozygosity observed in Huon pine however was not as low as has been observed in some other species of plants (e.g. Les *et al.* 1991).

At least three quarters of the Huon pine sites surveyed deviate significantly from Hardy-Weinberg expectations of genotypic frequencies. However, at least some of those not deviating from expectations, such as Mt. Read, have such low variation that few heterozygotes would be expected given the sample sizes. The Mt. Read population is unusual, as it appears to be principally derived clonally from very few individuals (see also previous chapter). Most stands have very high fixation indexes (Table 3.13), indicating high levels of inbreeding, the levels of which are comparable to other highly inbred species (Brown *et al.* 1975).

It is not surprising that most Huon pine stands are not in Hardy-Weinberg equilibrium since random mating is unlikely. For example on average only thirty percent of trees are likely to be reproductively active in a mast year and male to female ratios may or may not be even (Chapter 2). Furthermore the larger older trees dominate the seed and pollen output (Chapter 2), and stands are mostly small in size. Therefore, unless selected against, particular homozygous genotypes are likely to predominate. Selection against inbred individuals prevents inbreeding depression and increases heterozygosity, thus decreasing gene fixation in the selected population (Ennos 1989). Such selection favouring heterozygotes has been observed in other species (Coates 1992, Ennos 1989), including some with long life spans such as *Macrozamia riedlei* (Bryne and James 1991) and the palm *Astrocarum mexicanum* (Eguiarte *et al.* 1992). It seems to be found particularly in small populations where inbreeding may be less desirable (Ennos 1989). In cases such as populations

emerging from a bottleneck, there may be an observable increase in heterozygosity with age, as less fit inbred individuals are progressively selected against throughout the life span of the plant (Ennos 1989, Farris and Mitton 1984, Brown 1989). In Huon pine there was no evidence of selection against homozygotes and there was no evidence of selection favouring heterozygotes, as there was no increasing trend in the proportion of heterozygotes with increasing tree size (Figure 3.5).

Since Huon pine is mostly dioecious and therefore an obligate outcrosser, inbreeding is likely to be the result of biparental inbreeding between closely related individuals (Brown 1989). The vegetative reproductive habit of Huon pine will also contribute to the spread of particular genotypes and may help to maintain the higher levels of homozygotes found, leading to high fixation indexes. Biparental inbreeding usually occurs when there has been the development of family clusters, or microhabitat selection for particular genotypes (Brown 1989). Family clusters develop where there is poor gene flow (i.e. most seed falls beneath the maternal tree and pollination is mostly from nearest neighbours) (Epperson 1989). This counteracts the effect of new genotypes arising from sexually produced outcrossed offspring. Such a situation could develop in Huon pine as most seed falls beneath the maternal tree, particularly away from the banks (Chapter 1). Clonal reproduction could reinforce family substructuring and the allelic frequencies already present. Such genetic substructuring has been shown to have developed in some other species (Schoen and Latta 1989, Schnabel *et al.* 1991, Epperson 1989).

There was evidence, from spatial autocorrelation analysis, of the development of genetically homogeneous patches in all sites surveyed. The small and consistent size of the patches, and the finding (Chapter 2) that both sexes were clumped to the same degree in these sites, suggests that much of the substructuring is due to vegetative growth, although development of family structures appears to have contributed to some extent. The sites with the largest patch sizes (Greystone Bluff and Teepookana) however, showed less evidence of vegetative propagation. They were composed of more sparsely distributed trees away from watercourses, and had very high fixation indexes (Table 3.13). These attributes suggest that localised gene flow has been the major cause of genetic patch development in those sites.

Heterozygotes were less often significantly associated together than homozygotes. This may reflect the formation of heterozygote zones between mostly homozygous family groupings, rather than patch structure due to vegetative reproduction (Epperson 1989). The low frequency of heterozygotes prevents this from being

clarified. However, the study of site maps yielded results which would support this view.

Allelic fixation was not correlated to density (Table 3.11), as has been found in other species, where greater densities are thought to effectively localise gene flow (Loveless and Hamrick 1984, Farris and Mitton 1984). Interestingly, neither was fixation correlated to the proportion of reproductive trees nor to the proportion of females in the stands (Table 3.11). Gene flow between sites does not appear to be a major factor determining the amount of inbreeding, as sites fringing two major rivers where incoming seed and pollen should be greatest (Gordon River and Picton), were amongst the most inbred sites (Table 3.13). The results suggest that indeed most gene flow is highly localised and there is a lack of interaction between family or clonal groupings. However, occasional successful long distance wind pollination and seed dispersal may have acted in the long term to moderate the potential differentiation between sites to their current low levels. Ellstrand (1992) has suggested that effective gene flow (migration) of one individual per generation is sufficient to reduce inbreeding depression and loss of variation due to chance local losses in small populations. Thus, in Huon pine which has such a long generation time, rare past gene flow may have been sufficient to reduce allele loss and population differentiation and allow the species to be relatively homogeneous in its allelic composition over its range.

The limited variation in allelic composition within the species has also limited the amount of differentiation possible between sites (Table 3.6). Such limited variation has been observed in some other long lived species such as *Sequoiadendron giganteum* (Finns and Libby 1982), *Dacrydium cuppressinum* (Hawkins and Sweet 1989) and *Pinus resinosa* (Yeh 1981). These authors proposed that these species underwent a loss of variation due to severe bottlenecks during major past climatic changes. Such a scenario is also likely for Huon pine which has a long fossil record, during which there have been major fluctuations in its abundance and distribution (MacPhail 1979, Playford and Dettman 1979, Colhoun and Van de Geer 1987, Hill 1990). Huon pine populations would certainly have been greatly restricted during the Last Glacial which may have led to the loss of some less common allelic forms. There is evidence that some current populations have been continuously present since the Last Glacial and some may date back even earlier (Francey *et al.* 1984, Barbetti *et al* 1993). Relatively few generations may have passsed since the last glacial period, due to the longevity and persistence of the species. The Mt. Read site has been dated back to at least 11000 years (Barbetti *et al.* 1993). If, as has been

postulated, this site is mostly clonally derived, then the persistence of Huon pine trees/populations should not be underestimated.

Huon pine would appear to have a fairly conservative genetic structure, with its biology contributing to a resistance to change. For example older genotypes will tend to dominate offspring since the oldest, largest trees, are the greatest seed and pollen producers (Chapter 2). As shown above, vegetative reproduction can be significant, and will allow the persistence and dominance of old successful genotypic combinations. Therefore, unless strongly selected against, old genotypic combinations will tend to persist in this species. One of the interesting findings of the survey by Hamrick and Godt (1989) was that there was more genetic diversity among populations within species which are themselves members of large genera, and less diversity among populations of species from less diverse genera. This lends weight to the idea that some genera tend towards speciation (specialists), while others survive with more robust less variable genotypes (generalists) (Van Tienderen 1991, Hamrick *et al.* 1979). It could be speculated that the latter appears to be the case for Huon pine.

Lande (1988) suggests that several critical factors affect the persistence of subdivided populations, such as found in Huon pine. They include the number, size and spatial distribution of patches of suitable habitat, and the dispersal rates between subpopulations. There must be enough sites within dispersal distance, to reduce the negative effects of inbreeding, and enough potentially suitable sites, to allow for movement of populations should their present habitat become unsuitable. So for Huon pine survival, catchment integrity may be essential.

Huon pine populations are highly inbred due to vegetative reproduction, small populations and localised gene flow. This has lead to localised fixation of allelic frequencies which has arisen by drift or selection. There is low diversity among sites due to a narrow genetic base and possibly due to long distance seed and pollen dispersal. As there is no evidence for selection against homozygous genotypes, or favouring heterozygotes (Figure 3.5), while there is evidence for persistence of inbred stands (e.g. Mt. Read). Therefore inbreeding does not appear to present a major problem for the survival and fitness of Huon pine.

CHAPTER 4 :

Variation within and among *Atherosperma moschatum* populations.

INTRODUCTION

Atherosperma moschatum (sassafras) is the sole member of its genus. It is a member of the subfamily Atherospermatoideae within the Monimiaceae (Foreman 1984). There has been dispute as to the status of the Atherospermatoideae, with some authors giving it family status. However, there is general agreement that its members would have been the first to diverge from the rest of the family Monimiaceae (Foreman 1984). The members of the Monimiaceae are of interest as they may represent a transition in angiosperm evolution from the most primitive angiosperm families to more modern ones, since they have many putatively primitive characteristics. However, the tendency for unisexual flowers, such as occurs in *A. moschatum,* is considered to be a more advanced state (Foreman 1984). *A. moschatum* is poorly represented in the fossil record due to its tendency for rapid decay (Hill and Macphail 1985). The oldest record is a single fossil leaf which dates *Atherosperma* or a close relative, as present in the Tasmanian flora forty thousand years ago (Hill and Macphail 1985).

A. moschatum (sassafras) is the most widespread in geographic distribution of the Tasmanian temperate rainforest tree species (Hill *et al.* 1988). It is second in overall canopy abundance to *Nothofagus cunninghamii,* with which it is often codominant (Read 1985). Although most prolific in Tasmania, *A. moschatum* also occurs on mainland Australia (Hill *et al.* 1988). In mainland Australia *A. moschatum* is mostly found in Victoria where it is restricted to cool temperate rainforest and fire protected gullies in mixed forest, the largest populations being on the Errinundra Plateau (Floyd 1990). *A. moschatum* is notably absent from the cool temperate rainforests of the Otway ranges region (Hill *et al.* 1988). However, *A. moschatum* extends northwards into New South Wales in isolated pockets of cool temperate rainforest and mixed forest. It is found in fire protected south facing gullies and creek margins as far north as the Barrington Tops region, where it occurs at higher altitudes (Floyd 1989).

In Tasmania *A. moschatum* is found throughout the range of rainforest and mixed forest distribution, and is absent from only the high altitude rainforest sites. Sassafras

is a primary component of many of the small isolated rainforest fragments scattered through the east of the state. These are usually found in gullies amidst eucalypt dominated forests, where, particularly in the south, sassafras tends to be the dominant canopy species (Neyland 1991). These small populations are estimated to have persisted for at least several hundreds of years, in topographically fire protected areas, whilst the surrounding vegetation has burnt more frequently (Neyland 1991). Thus Neyland (1991) postulates that these rainforest relicts have remained small in size with little opportunity for expansion. Neyland (1991) also suggests that the conditions required for the rainforest to burn occur approximately once every five hundred years or more.

A. moschatum has seed which is easily wind dispersed, and it has been postulated that the small relict eastern Tasmanian stands, have been established or restocked after fires, by wind dispersed seed (Neyland 1991). Whilst the seed can potentially be dispersed widely, Read and Hill (1988) argued that within the closed rainforest canopy sassafras seed is poorly dispersed. *A. moschatum* is a prolific seed producer, with a large annual seed output (Hickey *et al.* 1982). Read and Hill (1988) have reported that regeneration is continuous, mostly by vegetative means, and that seedlings are rare in mature forests, even though they are reported to be shade tolerant (Read 1985). Neyland (1991) investigated seedling survival and found that sassafras germinated well under its own canopy, but the survival of seedlings was severely limited by browsing. Hickey *et al.* (1982), also suggested that browsing severely limited seedling establishment. Neyland (1991) pointed out that browsing did not account for all seedling loss and postulated that water stress, insect attack and fungal infection could all contribute to low seedling survival in *A. moschatum*. Read (1985) showed *A. moschatum* to be drought intolerant. Johnson and Lacey (1983) have suggested that vegetative reproduction is relatively common in cool temperate rainforest tree species, and that it enables these long-lived trees to persist and regenerate whilst bypassing the more vulnerable seedling stage.

There have been few studies which have investigated the genetics of natural populations of long-lived angiosperms (Hamrick and Godt 1989, Muona 1989), much less rainforest species (Hunziker and Schaal 1983, Gan *et al.* 1981, Sytsma and Schaal 1985, Equiarte *et al.* 1992, O'Malley *et al.* 1988). Most long lived species studied have been gymnosperms, which are mostly wind pollinated outcrossers and thus may be expected to show different levels and patterns of genetic variation to most angiosperm species (Hamrick *et al.* 1979). Few rainforest species have been studied in Australia (Moran *et al.* 1990), but eucalypt species have received

considerable attention (e.g. Moran and Bell 1983), as well as shrubs and herbs from drier habitats (e.g. Brown 1990, Coates 1988) .

The viability of temperate Tasmanian rainforest, especially its remnant patches, is largely determined by the survival of its canopy trees. Therefore an understanding of their biology is important for their long term management. *A. moschatum* is a major canopy species and often dominates in small relict patches and is thus a major habitat creator. Much regeneration in this species is reported to be vegetative (Read and Hill 1988). If so, this will have implications for its genetic variability and effective mode of breeding especially in small populations.

Isozyme analysis was used as the method of genetic analysis for this study since this method is well suited to large population studies of variation within a species (Brown 1990). This technique is at present the most rapid and cost effective method for genetic analysis of populations within species (Brown 1990). Both germination of seed and subsequent growth rates may be slow in *A. moschatum* (Read 1989). However, shoot material can be used for isozyme analysis, with the advantage that the actual rather than potential variation in a population can be assessed. Unfortunately when leaf material is used particularly from rainforest trees, fewer enzymes can be resolved for comparison than if seedling material is used, due to the secondary compounds present in the leaves (Stahman 1963, Loomis 1974).The practical advantages of independence from the use of seeds should not be underestimated however, and include the possibility to survey all age classes within a population.

The study investigated the nature of genetic variation in *A. moschatum* populations, throughout its geographical range. The relationships between the genetics, population dynamics and biology of *A. moschatum* were investigated. The project aimed to determine if small isolated populations were less diverse and more inbred than larger more continuous populations. The viability of small populations and implications of the studies findings for long term conservation of the species are discussed.

FIELD METHODS

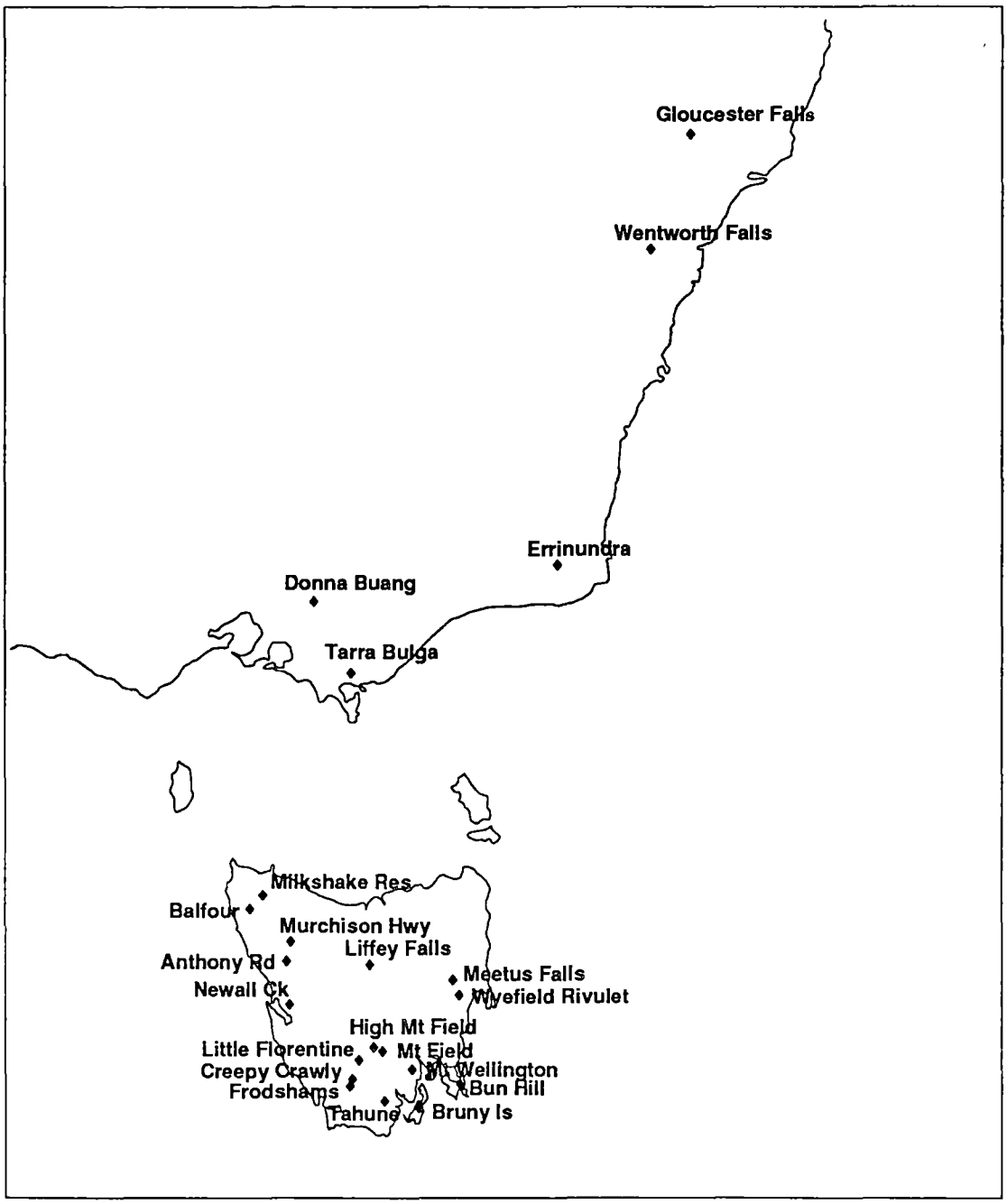
A survey of *Atherosperma moschatum* (sassafras) populations was undertaken (Figure 4.1). Twenty two sites were sampled for isozyme analysis of genetic variation. Sassafras trees were sampled along belt transects. At 19 sites all *A. moschatum* plants within the transect boundaries were noted, and given a unique

number, their height and diameter measured and their locations recorded as co-ordinates (length and breadth) relative to the transect line. All *A. moschatum* plants within these transects were sampled for isozyme analysis but only a sample of seedlings were collected for isozyme analysis if they were present in large numbers. However due to time constraints, samples only, were collected at three sites. Samples consisted of healthy mature leaves. Transects varied in length and breadth, depending on the size, shape and density of the stand sampled, and on the time available, between thirty and fifty samples were collected from most sites. Transects varied in length by multiples of the minimum length of thirty metres, and thirty metre transects were sometimes run parallel or perpendicular to each other (but not overlapping), rather than end to end. Transect width ranged from five to forty metres. In the case of multistemmed trees where it was clear that all shoots arose from the same base, the dominant stem was used for all measurements to be consistent between trees, but the number of other stems present was noted. If trees were unconnected to ground level they were treated as separate stems, though it was noted which trees might be connected below ground level if they were growing in clumps. Species lists of vascular plants were compiled for all Tasmanian study sites. Complete species lists were not compiled for the mainland sites. However species lists for these sites are available in the literature (Floyd 1990, Ashwell 1991).

ELECTROPHORETIC METHODS

Adult leaf material was used for electrophoretic analysis since seed production was unreliable and germination very slow, and since adult leaves give the current genetic composition of stands. Each sample for electrophoresis was placed into a sealed plastic bag. They were kept refrigerated on return from the field and within days frozen overnight (-20°C). Once frozen they were freeze dried as soon as possible given the limitations of freeze drier space (up to two weeks). The freeze dried samples were then stored in a sealed container in a freezer (-20°C) until used. Grinding and extraction of enzymes from leaves for electrophoresis must be done quickly to prevent enzyme denaturation. For population studies the techniques must also be suitable for processing large number of samples. The method used was an adaptation of the methods of several authors (Gan *et al.* 1981, Neale *et al.* .1984, Cheliak and Pitel 1984).

Figure 4.1 Map of the locations of *Atherosperma moschatum* study sites.



Each sample was ground to a coarse powder in liquid nitrogen with a mortar and pestle. Extraction buffer was mixed with the powder (see Table 4.1) and this mixture was squeezed through a course filter and absorbed onto filter paper (Whatman no.1) wicks. These were kept on ice until loaded onto the gel.

The extraction buffers used for woody plants are complex because they must counteract the effects of compounds such as phenols and tannins, which act in the ruptured cell to bind or otherwise inactivate enzymes. The buffers contain a range of additives which act to isolate and stabilise enzymes in a buffered solution. Most of the additives compete with the enzymes or prevent them from reacting with other compounds, such as phenols and tannins, by controlling or altering their chemical environment (Stahman 1963, Loomis 1974).

Table 4.1 Electrophoresis Extraction Buffer (modified from Cheliak W.M.&Pitel J.A. 1984)

STOCK SOLUTION	
	0.1M Phosphate buffer pH 6.8
	100g/1 Sucrose
	70g/1 Polyvinyl pyrrolidone (PVP) (40000mw)
	10g/1 PVP (360000mw)
per 50ml of stock solution add:	
	50mg Ascorbic acid (Na salt)
	85mg EDTA (di Na)
	300mg NaS_2O_5
	400mg Borax
	500mg Albumin (bovine serum)
	225mg DIECA
	50mg Dithiothrietol (DTT)
	10mg NADP
	20mg NAD

Samples were run on 12.5% starch gels using the methods described by Conkle *et al.* (1982). They were run under constant current conditions of 65 mA for 5 hours. A discontinuous Lithium Hydroxide, Tris, buffer system (Table 4.2) was used since it had been shown to give the best resolution of the bands after four standard systems had been tested (Moran and Bell 1983).

Table 4.2 Electrophoresis Gel and Tank Buffers (Brewbaker *et al.* 1968)

	Gel Buffer pH 8.2	0.065M Tris
		0.01M Citric acid
	Tank Buffer pH 8.5	0.05M LiOH
		0.19 M Boric acid
The gel is made of 9:1 mixture of gel:tank buffers.		

Table 4.3 Recipes for Enzyme Stains

IDH (Isocitrate Dehydrogenase) (E.C.1.1.1.42) (Shaw and Prasad 1970)

120ml H_2O
10ml 1.0 M Tris pH8.0
1ml $MgCl_2$ (10%)
50mg (Na) Isocitrate
32mg MTT
15mg PMS
10mg NADP

6PG (6-Phosphogluconate Dehydrogenase) (E.C. 1.1.1.1.44) (Brown *et al.* 1978)

117ml H_2O
10ml 1.0M Tris pH8.0
40mg 6-Phosphogluconic acid
32mg MTT
15mg PMS
10mg NADP

PER (Peroxidase) (E.C. 1.11.1.7) (Shaw and Prasad 1970)

150ml 0.2M Acetate, buffer pH 4.9
100 mg $CaCl_2$
1.5 ml 1% H_2O_2
50mg 3 - Amino - 9 ethyl carbazole
(dissolved in 5ml Dimethyl formamide)

AAT (Aspartate Aminotransferase) (E.C. 2.6.1.1) (Brown *et al.* 1978)

4ml 0.2M Phosphate buffer pH 4.9
250ml PVP
0.2 ml (0.05%) Pyridoxal - 5 -phosphate
1 ml (1.06%) α- ketoglutaric acid
1 ml (4.56%) Aspartic acid
50 mg Fast blue BB salt (in 2 ml H_2O)

GDH (Glutamate Dehydrogenase) E.C.1.4.1.2) (Shaw and Prasad 1970)

2g Glutamate (monosodium salt)
120 ml H_2O
10 ml 1.0 M Tris pH 8.0
32 mg MTT
15 mg PMS
10 mg NAD

SDH (Shikimic Dehydrogenase) E.C.1.1.1.25) (Moran and Hopper 1983)

120 ml H_2O
10 ml 1.0 M Tris pH 8.0
50 mg Shikimic acid
32 mg MTT
15 mg PMS
10 mg NADP

PGI (Phosphoglucose Isomerase) (E.C. 5.3.1.9) (Guries and Ledig 1978, Buth and Murphy 1980)

5ml H_2O
0.5 ml $MgCl_2$(10%)
1ml 1.0 M Tris pH 8.0
25mg Fructose - 6 - phosphate
10 units Glucose - 6 - phosphate dehydrogenase
16 mg MTT
7 mg PMS
5 mg NADP

Initially a wide variety of enzyme specific stains were screened to investigate those which produced clearly readable bands (IDH, MDH, FUM, GLY, 6PG, PGI, PER, G6PDH, GDH, AAT, ME, EST, PGM, AP, SDH). The following stains were chosen for further use. Glutamate dehydrogenase (GDH); Apartate aminotransferase (AAT); Shikimic dehydrogenase (SDH); 6-Phosphogluconate dehydrogenase (6PG); Isocritric dehydrogenase (IDH); Peroxidase (PER); Phosphoglucose isomerase (PGI). Stain recipes are given in Table 4.3.

Interpretation of Isozyme Banding Patterns

Samples from different sites were run on the same gels to provide cross references. Comparison of the full data set was undertaken, enabling the interpretation of the observed banding patterns into loci and alleles where observed patterns fitted expectations. Adult leaf material was used for isozyme analysis, therefore the allelic designations made could not be tested on progeny. Interpretations were compared with available literature for consistency (Gottlieb 1981 &1982, Neale *et al.* 1984, Moran and Bell 1983, Brown 1979, Richardson *et al.* 1986). Fresh material from Mt. Wellington and from potted specimens from Gloucester Tops was used during initial trial test runs. The results from freeze dried material was consistent with the results from the fresh material, indicating that significant breakdown of enzymes was not taking place (Richardson *et al.* 1986).

Aspartate aminotransferase (AAT)

In *A. moschatum* only one locus was consistently scored and was dominated by one allelic form(A), but three other alleles were also recorded (Figure 4.2). Heterozygotes appear to be represented as double bands only. AAT's are specified by three unlinked gene loci in most plants (Gottlieb 1982, Neale *et al.* 1984).They are usually eported to be dimeric (composed of two subunits), but interlocus heterodimers have not been observed (Gottlieb 1981).

Peroxidase (PER)

In *A. moschatum*, a few bands were present and these were consistently scorable, though they tended to be broad rather than sharp. Three allelic forms were recorded (Figure 4.2). Peroxidases oxidise a large number of hydrogen donors including phenolic substances (Gottlieb 1981), so it is not surprising that they are active in rainforest leaves. However, their electrophoretic patterns are often complex (Gottlieb 1981).

Plants usually have two isozymes for each enzyme of the glycolytic pathway, one in the cytoplasm and one in the chloroplast (Gottlieb 1982). It is not unusual however to find only one form of an enzyme and this usually indicates that different extraction and staining conditions are required for each to be resolved (Gottlieb 1982).

6 - Phosphoguconate Dehydrogenase (6PG)

Only one 'zone of activity' was found for this enzyme, consisting of a very thick band. It was thought that this represented one locus which was monomorphic, but a very rare, slightly faster allelic form (B) was observed in some populations (Figure 4.2).

Glutamate Dehydrogenase (GDH)

Only one locus was observed for this enzyme with three allelic forms. Heterozygotes were observed as single thick blurred intermediate bands. (Figure 4.2) This result is consistent with those of other authors (Moran & Bell 1983, Neale *et al.* 1984).

Isocitrate Dehydrogenase (IDH)

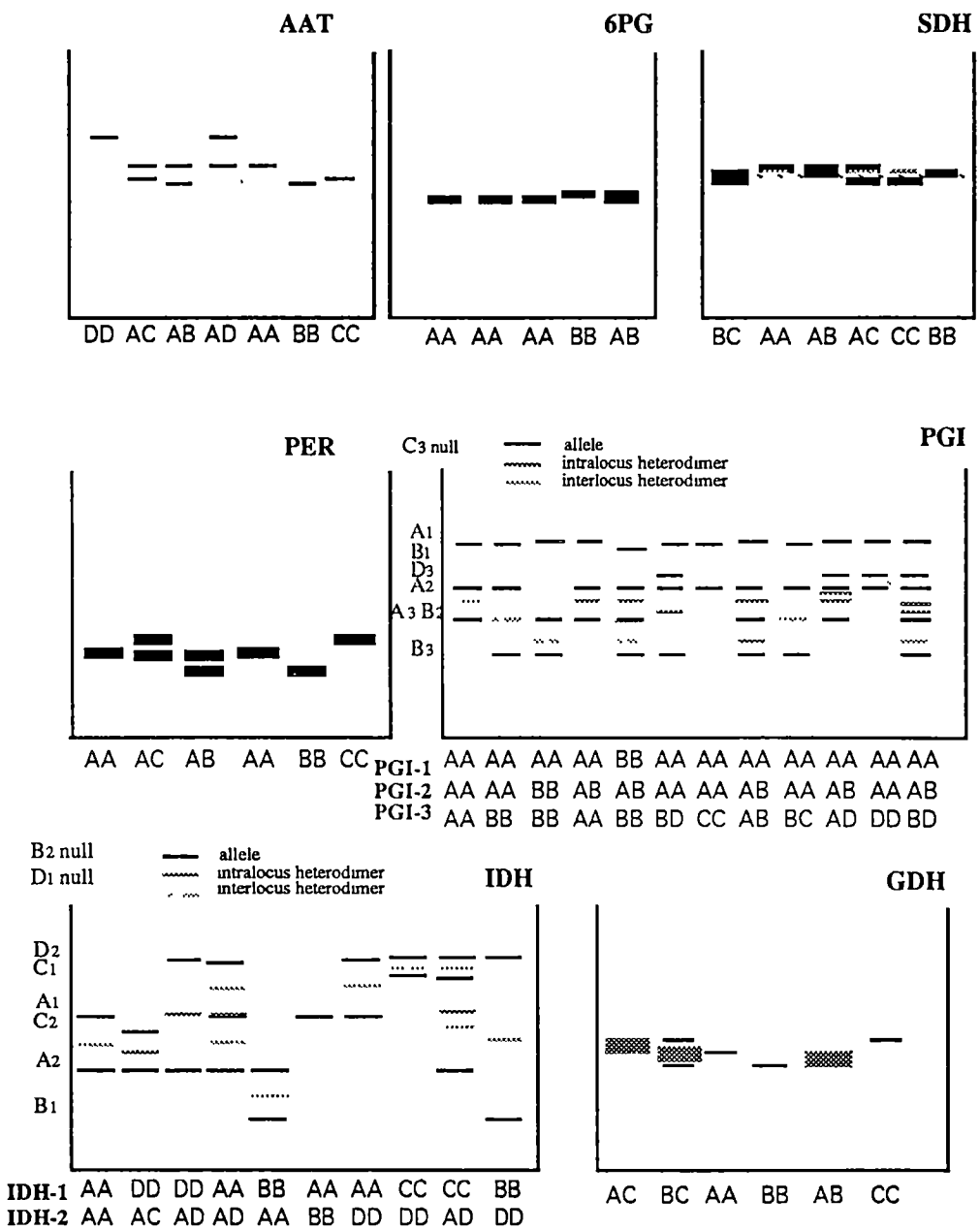
Two IDH loci were observed. IDH is reported to be a dimeric molecule (Neale *et al.* 1984), consequently triple banded heterozygotes are expected. In the Tasmanian populations trees were homozygous and invariant at each locus and in vitro interaction between the two enzymes resulted in three bands being recorded (Figure 4.2). More complex patterns resulted in the mainland populations where there was variation at each locus (Figure 4.2). Some allelic forms were expressed more weakly than others, suggesting that allelic difference may also reflect enzyme activity or expression. There appeared to be null allelic forms at each locus representing enzymes with reduced activity or expression. Interactions between loci were variable in their expression. The most simple interperetations which satisfied all the banding patterns observed and which were consistent with published results were used.

Shikimic Dehydrogenase (SDH)

In *A. moschatum* there appeared to be two loci present, but overlapping in migration distance. One of these was inconsistent and apparently invariant. It was excluded from the analysis since any variation would have been masked by the other more variable locus. There were three allelic forms present at this locus (Figure 4.2). All

the patterns observed, could be explained by this interpretation of loci and alleles. SDH is usually reported to be monomeric with a single zone of activity (Neale *et al.* 1984, Moran & Bell 1983).

Figure 4.2 The genotypic interpretation of the common isozyme banding patterns encountered in the analysis of seven enzyme systems in *Atherosperma moschatum* . The banding patterns are given for each enzyme and the genotype assigned given directly below each pattern. The thickness of bands given, is representational of the varying band thicknesses observed. Interlocus heterodimers were not always consistently observed, their positions are shown by lighter hatched bands, as indicated. Intralocus heterodimers are indicated by dark hatched bands, as indicated. The hatched bands on the SDH diagram indicate the position of a second, unscored and inconsistent locus. In PGI, as the two loci overlap in their mobility, there is occasional analogy of banding patterns. In such cases the frequency of alleles in the population and the intensity of bands were taken into account in final allelic designation. This may have resulted in slight under representation of the most uncommon heterozygous forms. * NB. the figure is not an exhaustive representation of all allellic combinations encountered.



Phosphoglucose Isomerase (PGI)

Three loci were observed. Two allelic forms were recorded in the fastest locus and these were not observed to form interlocus heterodimers. Two and four allelic forms were recorded in the other two loci which were observed to form both inter and intra heterodimers (Figure 4.2). These two slower loci overlapped in their migration (see Figure 4.2), thus some banding patterns may have analogous allelic interperetations (e.g. PGI AA BB BB~ PGI AA BB AB). In such cases, the most likely genotypes were scored taking into account the frequency of alleles in the population and the intensity of bands. Fortunately the potential frequency of overlapping genotypes was so low given the allelic frequencies that this is unlikely to significantly affect the results. This was the simplest designation of alleles and loci which satisfied all the banding patterns observed and which was consistent with published reports (Tanksley and Orton 1983, Gottlieb 1982). PGI is usually dimeric with enzymes in both plastids and cytosol. Some species have a duplicated locus producing two enzymes in the cytosol which may form interlocus heterodimers (Gottlieb 1982). Heterodimers are not usually observed between loci in the plastid and loci in the cytosol (Gottlieb 1982).

STATISTICAL METHODS

Diameter and Height

All *A. moschatum* plants scored were placed into diameter and height classes. These classes are given in Figures 4.3 and 4.4. The diameter sizes within each class increased by an increment with increasing diameter to take into account the distribution of diameter sizes found in this species. Numbers were converted to percentages of the population in each class to standardise for different sample sizes. The histograms generated were assessed visually. Density of sassafras trees was calculated from transect data.

Climatic Variables

The BIOCLIM program (Busby 1991) was used to generate sixteen synthetic climate variables for each site from the geographic co-ordinates and altitudes. These synthetic climatic variables were then included as site variables in a Principal Components Analysis (PCA) undertaken using the SAS/STAT ® procedure FACTOR (SAS Inst. Inc. 1990). The first principal components produced by the PCA analysis together account for 99.4% of the variation in the standardised data. These were treated as composite bioclimatic variables in further analyses.

Species

The DECODA statistical package was used to calculate a dissimilarity matrix based on species presence or absence using the Bray-Curtis distance measure (Minchin 1990). Hybrid Multi-dimensional scaling (Minchin 1987) was performed using this matrix with the PATN statistical package (Belbin 1991). A three dimensional solution was sufficient to give a stress value of 0.139. The output was plotted and this was used to visualise the affinities in species composition among sites.

Isozymes

The bands from each enzyme system were assigned to loci and genotypes and statistically analysed using the BIOSYS - 1 package of Swofford and Selander (1981). Allelic frequencies at each site were calculated and used to compute for each site; the mean number of alleles per locus (A), the mean percentage of polymorphic loci (P), the observed mean heterozygosity (Ho), and the expected mean heterozygosity under Hardy-Weinberg conditions (He), using Levene's (1949), correction for small sample sizes. Each population was tested for conformance of genotypic frequencies to those expected under Hardy-Weinberg equilibrium using a Chi-square goodness of fit test. The degree of inbreeding in populations was assessed by calculation of genotypic fixation indices (F) (Wright 1965) at each polymorphic locus where, F=(He-Ho)/He.

Genetic diversity between all sites was calculated using FST (Wright 1965) which is equivalent to Nei's (1973) GST (Nei 1977). Although developed from fixation indices this measure uses expected heterozygosity levels in its calculations and is thus independent of inbreeding. Populations were grouped according to geographic proximity (Table 4.4) and Wright's (1978) heirachical F statistics were used to analyse the partitioning of diversity and variance in this heirachy, i.e. between all populations, between regions and localities as well as between populations within regions and localities. The significance of interpopulation heterogeneity in allelic frequencies was evaluated by using a heterogeneity Chi-square test (Workman and Niswander 1970).

Similarity and distance coefficients were calculated between individual populations for each locus Rogers' 1972 (modified by Wright 1978) coefficient was chosen for futher use. Coefficients were also calculated across all loci for different levels in the

geographic hierarchy. An Unweighted Pair Group Method with Arithmetic Averaging (UPGMA) (Sneath and Sokal 1973) cluster analysis was performed on these matrices to obtain dendrograms. The goodness-of-fit of the dendrogram produced when compared to the input matrix, was evaluated using the co-phenetic correlation coefficient (Sneath and Sokal 1973), the F value of Prager and Wilson (1978), the Farris (1972) 'f' and the standard deviation (Fitch and Margoliash 1967). The cluster with the best fit was used for interpretation since all clusters fitted the data to a similar degree and clustered with similar groupings. A Distance Wagner dendrogram (Farris 1972) was produced using the genetic distance matrix produced, which gave similar groupings but with different interrelations.

Table 4. 4. Geographical heirarchy of *A. moschatum* sites used for genetic analysis.

	Population Heirarchy	
Regions	**MAINLAND**	
Locality	**NSW**	**VICTORIA**
Sites	WENTWORTH FALLS	DONNA BUANG
	GLOUCESTER FALLS	TARRA BULGA
		ERRINUNDRA
Regions	**WEST COAST**	
Locality	**SOUTH WEST**	**NORTH WEST**
Sites	NEWALL	MURCHISON HWY
	ANTHONY RD	MILKSHAKE RES
		BALFOUR TRACK
		LIFFEY FALLS
Regions	**CENTRAL SOUTH**	
Locality	**E CENT SOUTH**	**W CENT SOUTH**
Sites	MT FIELD	LIT FLORENTINE
	HIGH MT FIELD	CREEPY CRAWLY
		FRODSHAMS
Regions	**EAST COAST**	
Locality	**NORTH EAST**	**SOUTH EAST**
Sites	MEETUS FALLS	MT WELLINGTON
	WYEFIELD RIVLET	BUN HILL
		BRUNY ISLAND
		TAHUNE

Relationships Between Site Variables

Dissimilarity matrices were generated for several variable types. Geographic grid coordinates were used as the variables to generate a geographic distance matrix using squared Euclidean distance. Squared Euclidean distances were used as the distance measures to generate dissimilarity matrices from sassafras tree density, and

a composite climatic distance matrix, generated from the four PCA/BIOCLIM synthetic variables (see above). The PATN statistical package (Belbin 1991) was used to compute these distance matrices. The DECODA statistical package was used to calculate a dissimilarity matrix based on species presence or absence using the Bray-Curtis distance measure (Minchin 1990). Genetic distance was calculated on the BIOSYS-1 statistical package of Swofford and Selander (1981) using Roger's 1972 (modified by Wright 1978) measure of genetic distance. These dissimilarity matrices were paired in all possible combinations and analysed for correlations using the Mantels test (1967), which was undertaken on a program based on that published by Manley (1985).

RESULTS

Physical Structure of Sites

Diameter and Height

Generally there was a fairly even spread of trees throughout the range of diameter size classes with Mt Wellington having the most even distribution (Figure 4.3). Many sites had distributions of trees with a decline in the larger diameter classes. Eight sites however, had broadly pulsed distributions with smaller percentages of trees in both extremes of the distribution (i.e. Milkshake Res, Meetus Falls, Errinundra, Wyefield rivulet, Little Florentine, Mt Field, Bun Hill and Tahune). The negative exponential model of growth (Hett and Loucks 1976) does not appropriately describe diameter size distribution for these sites. Only the Newall Creek and Bruny Island sites broadly approximate this kind of distribution. Some of the sites appear to have had more than one pulse of past regeneration (e.g. Liffey Falls, Errinundra, Tarra-Bulga, Anthony Rd) see Figure 4.3. In general at most sites the current diameter distributions do not indicate much new regeneration. The Creepy Crawly site recorded the trees with the largest girths.

The tallest trees were recorded at Liffey Falls and the High Mt Field site had the lowest canopy height (Figure 4.4). The height distributions roughly reflected the diameter distributions. However, in many cases there was some compression of the spread, so that for example trees tended to reach canopy height at smaller diameters and remain there at larger diameters (Figure 4.4). In sites such as Tarra-Bulga there was a marked deficiency of medium height trees a similar lack of medium sized trees was not detected in the diameter distribution (Figures 4.3 &4.4).

Density

The Wentworth falls site had the greatest density of trees (Table 4.5), however this was a very atypical site and the trees were all very small in both height, diameter and form. Wyefield Rivulet, Murchison Highway and Frodshams Pass sites were the most dense Tasmanian stands and more typical of sites generally (Table 4.5). These stands had open understories and were dominated by medium sized sassafras trees which tended to occur in clusters (Figures 4.3 & 4.4). The least dense stands occurred at High Mt Field, Tarra Bulga and Donna Buang (Table 4.5). The High Mt Field site consisted of small clumps of small sassafras trees in sheltered sites, following a creek line amongst a boulder scree, whereas the Tarra Bulga and Donna Buang sites consisted of larger trees widely spaced in protected gullies.

Table 4.5. Transect area and *Atherosperma moschatum* density at each site .

Pop No	Code	Population	Density (Stems/m^2)	Transect Area (m^2)	No Trees in transect	Ave Dist (m) Between Trees
1	MFS	MT FIELD	0.3060	1470	45	3.2
2	MFH	HIGH MT FIELD	0.0027	9000	25	10.7
3	MWS	MT WELLINGTON	0.0145	3982	58	4.7
4	BHS	BUN HILL	0.0258	2400	62	3.5
5	LFS	LITTLE FLORENTINE	0.0178	1400	25	4.2
6	CCS	CREEPY CRAWLY	0.0165	3640	60	4.4
7	FSS	FRODSHAMS	0.0667	660	44	2.2
8	BIS	BRUNY ISLAND	0.0148	1690	25	4.6
9	MUF	MEETUS FALLS	0.0321	1560	50	3.2
10	WRS	WYEFIELD RIVULET	0.0920	750	69	1.9
11	THS	TAHUNE	0.0115	3640	42	5.3
12	DBS	DONNA BUANG	0.0060	20000	120	7.3
13	TBS	TARRA BULGA	0.0059	5025	30	7.3
14	ERS	ERRINUNDRA	0.0333	900	30	3.1
15	WFS	WENTWORTH FALLS	0.2667	150	40	1.1
16	GFS	GLOUCESTER FALLS	0.0500	800	40	2.5
17	NAS	NEWALL CK	0.0260	2160	56	3.5
18	ARS	ANTHONY RD	0.0250	1350	34	3.6
19	MHS	MURCHISON HWY	0.0950	600	57	1.8
20	MRS	MILKSHAKE RES	0.0225	2400	54	3.8
21	BTS	BALFOUR TRACK	0.0129	2400	31	5.0
22	LIF	LIFFEY FALLS	0.0093	6450	60	5.9

Species Composition

The mainland sites were often quite distinct from the Tasmanian populations in their species composition since many species in these forests do not occur in Tasmania. Each site had different codominant canopy species present. The Victorian Tarra-Bulga site was the mainland community most like the Tasmanian sassafras communities. The NSW sites were very different to the Tasmanian communities (See Appendix 2). Descriptions of the species compositions of these sites and their community types are available in Floyd (1990) and Ashwell (1991).The

Figure 4.3 The diameter distribution of *Atherosperma moschatum* plants at each site. The percentage of plants in each diameter class is plotted.

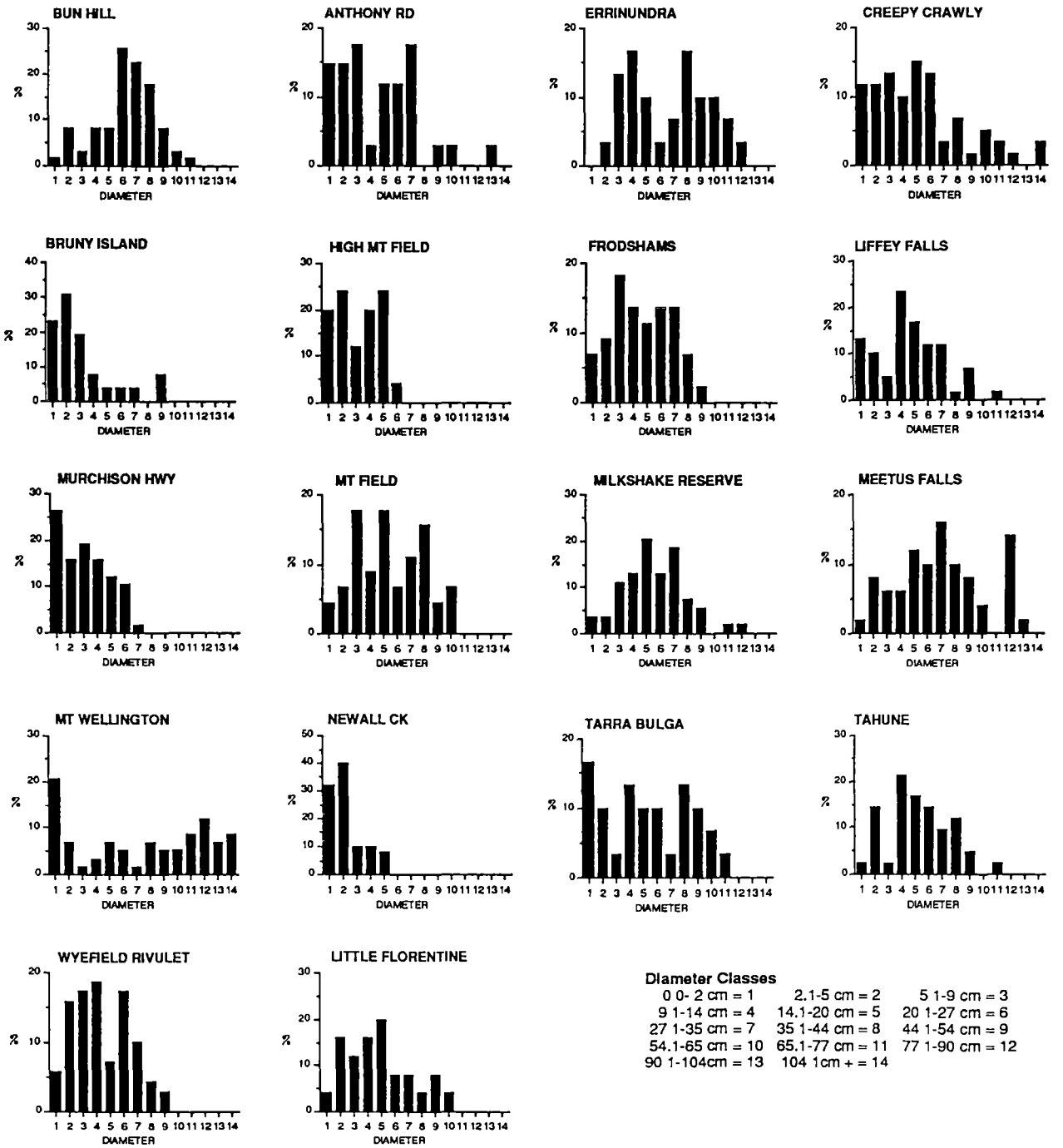
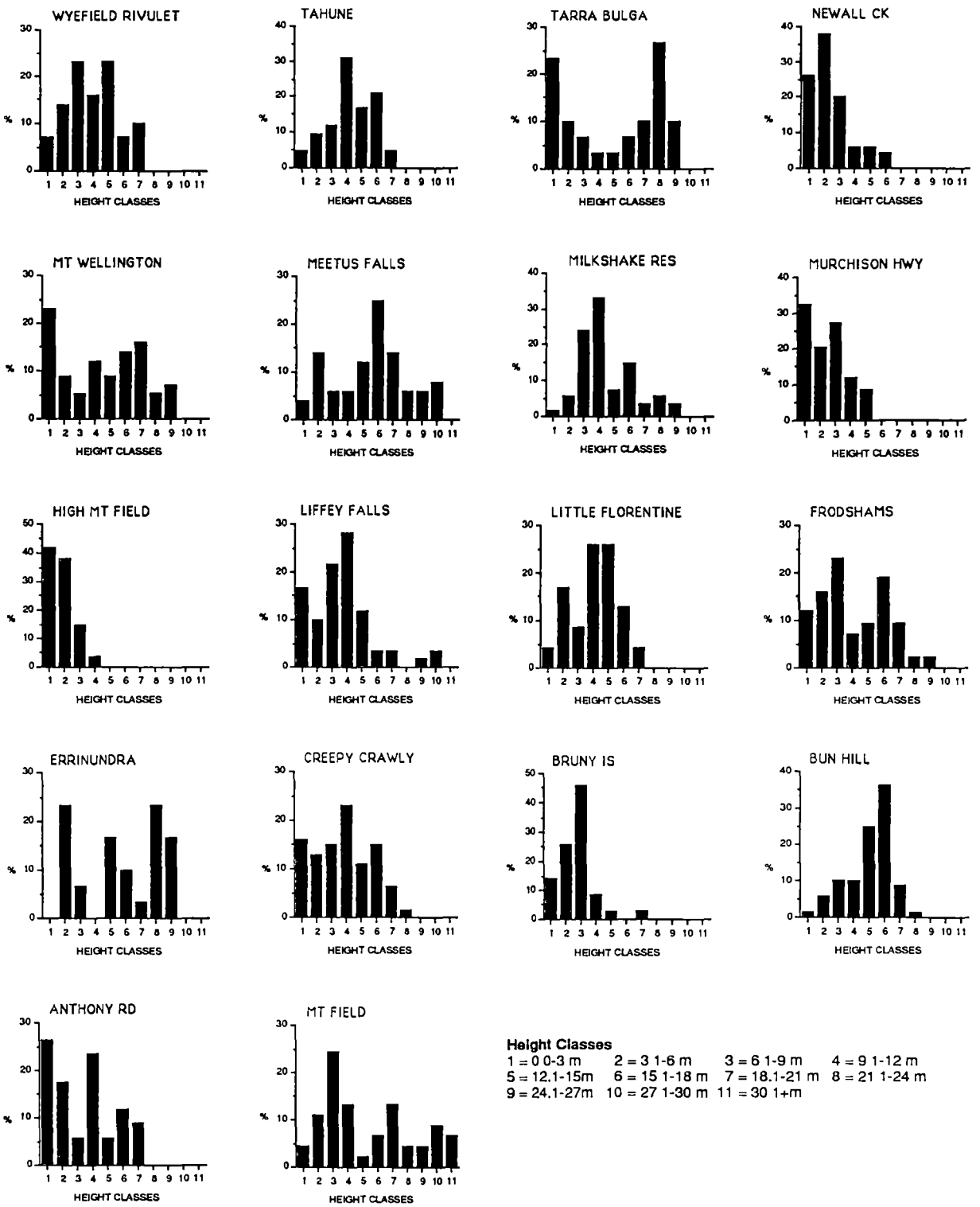


Figure 4.4 Height distributions of *Atherosperma moschatum* plants at each site.

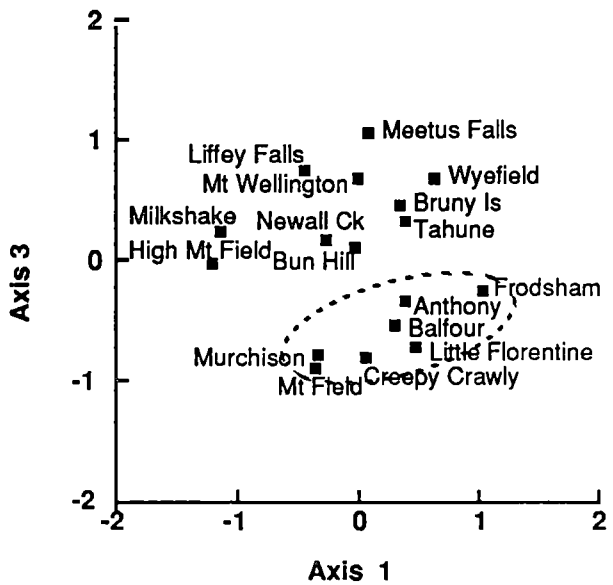


Height Classes

1 = 0 0-3 m 2 = 3 1-6 m 3 = 6 1-9 m 4 = 9 1-12 m
5 = 12.1-15m 6 = 15 1-18 m 7 = 18.1-21 m 8 = 21 1-24 m
9 = 24.1-27m 10 = 27 1-30 m 11 = 30 1+m

Tasmanian sites when analysed for species composition similarity, reflected the ecological diversity of the sites selected for study. The most similar sites roughly formed a group which corresponded to sites within callidendrous (Jarman *et al.* 1984) rainforest areas which had lower species diversity and more open understoreys (Mt Field, Anthony Rd, Murchison Hwy, Little Florentine, Creepy Crawly and Balfour) (Figure 4.5). These sites are little affected by species more usually associated with different surrounding vegetation types.

Figure 4.5 Plot of species composition affinities among the study sites. The plot is produced from two axes resulting from a Hybrid Mulidimensional Scaling analysis performed on a Bray-Curtis dissimilarity matrix of species presence or absence.The most similar sites are grouped.



Population Genetics

Diversity and Breeding

Genetic diversity varied between sites. Some loci which were invariant in Tasmania were polymorphic in mainland populations (Table 4.6). Overall the proportion of polymorphic loci at each site varied from 50 to 80%, and the average number of alleles per locus varied from 1.6 to 2.4 (Table 4.7). The Little Florentine and Meetus Falls sites had the lowest percentages of polymorphic loci (50%) and the lowest average number of alleles per locus (1.6 & 1.7). However, the Little Florentine site also had a small sample size (Table 4.7) which may have biased these results. The Donna Buang and Murchison Highway sites had the greatest average number of alleles per locus (2.4) and were amongst those with the highest proportions of polymorphic loci (80%) (Table 4.7).

Table 4.6. Allele frequencies at each *A. moschatum* site.

		POPULATION CODE																	Vic Sites			NSW Sites	
Enzyme	Allele	MFS	MFH	MWS	BHS	BIS	MUF	WRS	THS	LFS	CCS	FSS	NAS	ARS	MHS	MRS	BTS	LIF	DBS	TBS	ERS	WFS	GFS
AAT	(N)	39	24	54	60	26	44	61	41	20	55	35	45	31	54	50	31	53	46	27	32	36	35
	A	0.962	0.938	0.963	0.817	0.846	1	0.721	0.707	1	0.9	0.957	0.922	0.871	0.944	1	0.903	0.906	0.859	0.944	0.969	0.764	0.929
	B	0.013	0.042	0.019	0.083	0.096	0	0.172	0.28	0	0.082	0	0.011	0	0.019	0	0	0.038	0	0	0	0	0
	C	0.026	0.021	0.019	0.1	0.058	0	0.057	0	0	0.018	0.043	0.067	0.129	0.009	0	0.032	0.009	0	0	0	0	0
	D	0	0	0	0	0	0	0.049	0.012	0	0	0	0	0	0.028	0	0.065	0.047	0.141	0.056	0.031	0.236	0.071
GDH	(N)	39	24	54	61	26	44	61	41	20	54	35	45	31	54	50	31	53	46	27	32	36	35
	A	0.372	0.604	0.157	0.336	0.269	0.477	0.525	0.305	0.4	0.167	0.486	0.433	0 597	0 519	0.54	0.581	0.387	0.446	0.278	0.578	0	0
	B	0.628	0.396	0.843	0.664	0.731	0.523	0.475	0.695	0.6	0.833	0.486	0.567	0.403	0.444	0.46	0.419	0.613	0.272	0.074	0.313	0	0
	C	0	0	0	0	0	0	0	0	0	0	0.029	0	0	0 037	0	0	0	0.283	0.648	0.109	1	1
6PG	(N)	39	24	54	61	26	44	61	41	20	55	35	45	31	54	50	31	53	46	27	32	36	35
	A	1	0.708	0.991	0.984	1	1	1	1	1	0.927	1	1	1	0.944	1	1	1	1	1	1	1	1
	B	0	0.292	0.009	0.016	0	0	0	0	0	0.073	0	0	0	0.056	0	0	0	0	0	0	0	0
SDH	(N)	39	24	48	53	22	35	60	41	12	55	35	45	31	54	50	31	53	46	27	32	36	35
	A	0.295	0.729	0.385	0.651	0.614	0.229	0.658	0.756	0.917	0.873	0.529	0.511	0 581	0 417	0.36	0.306	0.453	0 728	0.963	0 75	0.639	0 314
	B	0.513	0	0.375	0	0.386	0	0.058	0.073	0	0	0	0.089	0 032	0 296	0.17	0.129	0 019	0.207	0.037	0.078	0.139	0.1
	C	0.192	0.271	0.24	0.349	0	0.771	0.283	0.171	0.083	0.127	0.471	0.4	0 387	0.287	0.47	0.565	0.528	0.065	0	0 172	0 222	0.586
PER	(N)	39	24	54	61	26	44	60	41	20	55	35	45	31	54	50	31	53	46	27	32	36	35
	A	0.897	0.854	0.935	0.779	0.75	0.591	0.583	0.671	0.65	0.709	0.586	0.389	0.306	0.62	0.47	0.371	0 547	0.511	0.759	0 891	0.611	0.543
	B	0.103	0.146	0.065	0.221	0.25	0.409	0.417	0.28	0.35	0.291	0.414	0 611	0.694	0.38	0 52	0 629	0 453	0.489	0.204	0.109	0.361	0.4
	C	0	0	0	0	0	0	0	0.049	0	0	0	0	0	0	0.01	0	0	0	0.037	0	0 028	0.057
IDH-1	(N)	39	24	54	61	26	44	61	41	20	55	35	45	31	54	50	31	53	46	27	32	36	35
	A	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0 87	0.944	0 813	0.819	0.714
	B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.188	0 139	0
	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0 011	0.037	0	0	0.286
	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0 12	0.019	0	0.042	0
IDH-2	(N)	39	24	54	61	26	44	61	41	20	55	35	45	31	54	50	31	53	46	27	32	36	35
	A	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.837	0 889	0.875	0 875	0.771
	B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0 022	0.019	0	0	0.171
	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.098	0	0	0.014	0
	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.043	0.093	0.125	0 111	0.057
PGI-1	(N)	39	24	54	61	26	44	61	41	20	54	35	42	31	53	50	31	52	46	27	32	36	34
	A	1	1	1	1	1	1	1	1	1	1	1	0.714	1	0 708	0.67	0.726	0 904	1	1	1	0 889	0.5
	B	0	0	0	0	0	0	0	0	0	0	0	0.286	0	0.292	0.33	0 274	0.096	0	0	0	0.111	0.5
PGI-2	(N)	38	24	54	56	26	44	59	39	20	49	30	42	30	53	50	31	52	46	27	31	36	34
	A	0.711	0.521	0.648	0.616	0.481	0.693	0.542	0.731	0.325	0.735	0.6	0.512	0.817	0.717	0.69	0.903	0.663	0.587	0.907	0 935	1	0.794
	B	0.289	0.479	0.352	0.384	0.519	0.307	0.458	0.269	0.675	0.265	0.4	0.488	0.183	0 283	0.31	0 097	0 337	0.413	0.093	0.065	0	0.206
PGI-3	(N)	38	24	54	56	26	44	59	39	20	49	30	42	30	53	50	31	52	46	27	31	36	34
	A	0.368	0.167	0.38	0.446	0.5	0.625	0.551	0.051	0.175	0.653	0.683	0 369	0 45	0.528	0.43	0 29	0.356	0.587	0.519	0 419	0.361	0.309
	B	0.342	0.75	0.38	0.518	0.5	0.307	0.407	0.949	0.775	0.102	0.15	0.476	0 267	0.274	0 28	0.258	0 365	0.348	0.296	0 226	0.125	0.529
	C	0 289	0.083	0.241	0.036	0	0.045	0.034	0	0.05	0.245	0.167	0.024	0	0.038	0 02	0.097	0 019	0.065	0.185	0 355	0.514	0.162
	D	0	0	0	0	0	0.023	0.008	0	0	0	0	0.131	0.283	0.16	0 27	0.355	0.26	0	0	0	0	0

Site codes: MFS- Mt Field; MFH- Mt Field High; MWS- Mt Wellington; BHS- Bun Hill; LFS- Little Florentine, CCS- Creepy Crawly; FSS- Frodshams, BIS- Bruny Is; MUF- Meetus Falls; WRS- Wyefield Riv; THS- Tahune; NAS- Newall Ck; ARS- Anthony Rd; MHS- Murchison Hwy; MRS- Milkshake Res; BTS- Balfour Track; LIF- Liffey Falls; DBS- Donna Buang; TBS- Tarra Bulga, ERS- Errinundra; WFS- Wentworth Falls; GFS- Gloucester Falls.
(N) - Sample size; A, B, C, D are allelic designations.

Table 4.7. Summary of genetic variability measures averaged over all loci in *Atherosperma moschatum* populations.

Population	Mean sample size per Locus	Mean no. of alleles per locus	Percentage of loci polymorphic*	Mean Heterozygosity Direct-count	Mean Heterozygosity HdyWbg expected**
BUN HILL	59.1	2.0 (0.9)	80 (-0.2)	0.149 (-0.049)	0.265 (-0.071)
BRUNY ISLAND	25.6	1.7 (0.4)	60 (-0.2)	0.087 (-0.0410	0.256 (-0.073)
MEETUS FALLS	43.1	1.7 (0.9)	50 (-0.3)	0.118 (-0.05)	0.23 (-0.078)
WYEFIELD RIVULET	60.4	2.1 (0.3)	60 (-0.4)	0.138 (-0.042)	0.296 (-0.081)
TAHUNE	40.6	2.0 (0.3)	70 (-0.3)	0.07 (-0.021)	0.225 (-0.068)
MT WELLINGTON	53.4	2.0 (0.6)	70 (-0.3)	0.043 (-0.014)	0.226 (-0.086)
MT FIELD	38.8	1.9 (0.1)	60 (-0.3)	0.028 (-0.013)	0.245 (-0.087)
FRODSHAMS	34	1.8 (0.7)	60 (-0.2)	0.126 (-0.05)	0.26 (-0.081)
HIGH MT FIELD	24	1.9 (0.0)	70 (-0.2)	0.137 (-0.047)	0.261 (-0.067)
LIT FLORENTINE	19.2	1.6 (0.8)	50 (-0.2)	0.057 (-0.024)	0.194 (-0.071)
CREEPY CRAWLY	53.6	1.9 (0.8)	70 (-0.2)	0.083 (-0.025)	0.214 (-0.059)
NEWALL	44.1	2.1 (0.5)	70 (-0.3)	0.16 (-0.057)	0.325 (-0.081)
ANTHONY RD	30.8	1.8 (0.1)	60 (-0.2)	0.163 (-0.058)	0.263 (-0.08)
MURCHISON HWY	53.7	2.4 (0.2)	80 (-0.30	0.127 (-0.038)	0.334 (-0.081)
MILKSHAKE RES	50	2.0 (0.0)	60 (-0.3)	0.14 (-0.051)	0.319 (-0.09)
LIFFEY FALLS	52.7	2.2 (0.2)	70 (-0.4)	0.143 (-0.048)	0.298 (-0.081)
BALFOUR TRACK	31	2.1 (0.0)	70 (-0.3)	0.184 (-0.059)	0.304 (-0.084)
DONNA BUANG	46	2.4 (0.0)	80 (-0.3)	0.141 (-0.033)	0.338 (-0.071)
TARRA BULGA	27	2.3 (0.0)	80 (-0.3)	0.067 (-0.013)	0.218 (-0.068)
ERRINUNDRA	31.8	2.1 (0.1)	80 (-0.2)	0.044 (-0.014)	0.254 (-0.073)
WENTWORTH FALLS	36	2.2 (0.0)	70 (-0.3)	0.081 (-0.024)	0.273 (-0.072)
GLOUCESTER FALLS	34.7	2.2 (0.2)	80 (-0.2)	0.072 (-0.031)	0.348 (-0.072)
WEIGHTED MEAN	40.4	2.0	68	0.118	0.277

* A locus is considered polymorphic if more than one allele was detected.

** Unbiased estimate (see Nei, 1978)

(standard errors in parentheses)

Generally genotypic frequencies differed significantly from Hardy-Weinberg expectations (Table 4.8). Of the six enzymes that were most consistently variable, genotypes were in proportions deviating significantly from Hardy-Weinberg expectations in 69% of all cases (Table 4.8). The Balfour track site deviated least, with genotypic proportions significantly deviating from expectations in only two out of six enzymes. As may be expected from these results, the mean heterozygosity across all sites (0.118) was less than half that expected under Hardy-Weinberg conditions (Table 4.7).

Table 4.8. The significance of deviation from Hardy-Weinberg expectations of *A. moschatum* populations for each of the variable enzyme loci ($p < 0.001$). * $p < 0.05$ NS given when population is not variable, as is does not deviate from expectations.

POP No	POPULATION	AAT	GDH	SDH	PER	PGI2	PGI3
1	MT FIELD	NS	SIG	SIG	SIG	SIG	SIG
2	HIGH MT FIELD	NS	NS	SIG	SIG	SIG	SIG
3	MT WELLINGTON	NS	SIG	SIG	SIG	SIG	SIG
4	BUN HILL	SIG*	NS	SIG	SIG	SIG	SIG
5	LITTLE FLORENTINE	NS	SIG	NS	SIG	SIG	SIG
6	CREEPY CRAWLY	SIG	SIG	NS	SIG	SIG	SIG
7	FRODSHAMS	NS	SIG	NS	SIG	NS	SIG
8	BRUNY ISLAND	SIG	SIG	NS	SIG	SIG	SIG
9	MEETUS FALLS	NS	SIG	NS	SIG	SIG	SIG
10	WYEFIELD RIVULET	SIG	SIG	SIG	SIG	SIG	SIG
11	TAHUNE	SIG	SIG	SIG	SIG	SIG	NS
12	DONNA BUANG	NS	SIG	SIG	SIG	SIG*	SIG
13	TARRA BULGA	NS	SIG	SIG	SIG	NS	SIG
14	ERRINUNDRA	NS	SIG	SIG	SIG	NS	SIG
15	WENTWORTH FALLS	SIG	NS	SIG	SIG	SIG	SIG
16	GLOUCESTER FALLS	NS	NS	SIG	SIG	SIG	SIG
17	NEWALL CK	NS	NS	SIG	SIG	SIG	SIG
18	ANTHONY RD	NS	NS	SIG	SIG	SIG*	SIG
19	MURCHISON HWY	NS	SIG	SIG	SIG	SIG	SIG
20	MILKSHAKE RESERVE	NS	SIG*	SIG	SIG	SIG	SIG
21	BALFOUR TRACK	NS	NS	SIG	NS	NS	SIG
22	LIFFEY FALLS	NS	NS	SIG	SIG	SIG	SIG

The fixation index (Wright 1965) is used as a measure of inbreeding. Generally there was a high degree of allelic fixation in populations consistent with low levels of heterozygosity and indicating inbreeding. The fixation indices for these populations varied both among sites and enzymes. The enzymes least often 'fixed', and most variable in their F values were AAT, GDH and SDH (Table 4.9). The variability of IDH enzymes in mainland sites set them apart from Tasmanian populations, which were fixed for single alleles (Table 4.6). Wrights (1965) FST, based on fixation indices, is equivalent to Nei's (1973) GST and is a measure of gene diversity among sites. However, since it uses allelic frequencies it is independent of the degree of

inbreeding among sites (Nei 1977). The FST calculated for sassafras indicated fairly low diversity among sites 0.178 (Table 4.10), and F statistics (Wright 1965) (Table 4.10), indicated that most of the variation found in *A. moschatum* is accounted for by variation between individuals within sites. The results of hierarchical F statistical analysis (Wright 1978) between geographic groupings of sites indicate that the variation among sites within localities is approximately equivalent to the variation among localities, but approximately double the variation among localities within regions or the variation among regions (Table 4.11). This suggests that there is some regional differentiation among sassafras sites, for example mainland from Tasmania but also some local differentiation, such as among the Little Florentine, Frodshams and Creepy Crawly sites.

Table 4.9. Wrights (1965) fixation indexes for heterozygote deficiences or excesses for each enzyme in *A. moschatum* populations. (1 = Fixed, 0 = Hardy - Weinberg equalibrium,-1 = Heterozygote excess) * No enzyme variation.

POPULATION	AAT	GDH	SDH	PER	PGI2	PGI3
MT FIELD	-0.03	0.84	0.96	1.00	0.74	1.00
HIGH MT FIELD	-0.05	0.22	0.68	0.83	0.75	0.59
MT WELLINGTON	-0.03	0.65	0.97	0.85	0.76	0.86
BUN HILL	0.21	-0.07	0.54	0.57	0.59	0.60
LITTLE FLORENTINE	*	0.58	-0.09	0.78	0.89	0.86
CREEPY CRAWLY	0.50	0.47	0.18	0.56	0.79	0.72
FRODSHAMS	-0.05	0.89	0.14	0.47	0.31	0.79
BRUNY ISLAND	0.72	0.81	0.14	0.49	0.92	0.85
MEETUS FALLS	*	0.55	-0.30	0.62	0.41	0.87
WYEFIELD RIVULET	0.48	0.34	0.34	0.52	0.73	0.74
TAHUNE	0.65	0.94	0.75	0.74	0.54	-0.05
DONNA BUANG	0.19	0.83	0.54	0.78	0.37	0.75
TARRA BULGA	-0.06	0.85	-0.04	0.71	0.34	0.88
ERRINUNDRA	-0.03	0.94	0.84	0.52	0.47	1.00
WENTWORTH FALLS	0.46	*	0.74	0.78	*	0.77
GLOUCESTER FALLS	0.35	*	0.90	0.47	1.00	0.71
NEWALL CK	0.23	0.14	0.61	0.77	0.48	0.23
ANTHONY RD	-0.15	-0.14	0.75	0.62	0.44	0.43
MURCHISON HWY	-0.04	0.37	0.58	0.73	0.72	0.60
MILKSHAKE RESERVE	*	0.28	0.58	0.69	0.58	0.37
BALFOUR TRACK	-0.08	-0.06	0.83	0.38	-0.11	0.36
LIFFEY FALLS	0.04	0.17	0.71	0.70	0.61	0.43

Differentiation of Mainland populations

The mainland populations differed from Tasmanian populations by the presence of allelic variants at five loci (Table 4.6). The Tasmanian populations were monoallelic for both IDH loci, whereas a further three alternative alleles were found in mainland populations at both loci. Not all allelic forms were present at every site. The GDH allelic variant (C) was present in all mainland sites but only found in two Tasmanian sites (Frodshams and Murchison Hwy). This was the only GDH form present in the

N.S.W. sites. Two minor allelic forms (B&C) of AAT were not found in the Mainland sites, whereas at least one of these was present in all except two Tasmanian populations.

The N.S.W. populations were most like the Victorian Tarra -Bulga site, sharing the occurrence of a further PER allele (C), which was only found in two other sites, and the dominance of the otherwise rare GDH (C) allelic form. However, the total absence of the common GDH allelic forms (A&B) found in all other sites set the N.S.W. sites apart from all others. Wentworth falls was the only site with no PGI-2 (B) allele. The Gloucester falls population was further differentiated by having much higher proportions of IDH-1 (C), otherwise found only in low frequencies in two Victorian sites (Donna Buang and Tarra Bulga), and the otherwise uncommon PGI-1 (B) allelic form, absent in Victorian sites (Table 4.6).

Table 4.10. Summary of Wright's (1965) F statistics for all loci averaged over all *A. moschatum* sites studied and averaged over all loci. The statistics partition the genetic diversity into its components within and among sites.

Locus	F(IS)	F(IT)	F(ST)
AAT	0.287	0.349	0.086
GDH	0.483	0.635	0.295
6PG	0.329	0.459	0.194
SDH	0.594	0.669	0.184
PER	0.646	0.690	0.124
IDH 1	0.832	0.860	0.168
IDH 2	0.449	0.510	0.111
PGI 1	0.946	0.961	0.267
PGI 2	0.618	0.664	0.120
PGI 3	0.670	0.720	0.152
Mean	0.598	0.670	0.178

F(IS) Diversity among individuals within sites.
F(IT) Diversity among individuals within the total.
F(ST) Diversity among sites within the total .

Table 4 11 . Heirachical F statistics (Wright 1978) combined across all loci for *A. moschatum* populations analyse the partitioning of genetic diversity among sites.

Among X	Within Y	Variance component	Diversity F(XY)
Sites	Locality	0.299	0.100
Sites	Regions	0.424	0.136
Sites	Total	0.541	0.167
Locality	Regions	0.125	0.040
Locality	Total	0.242	0.075
Regions	Total	0.117	0.036

Genetic Distance

Genetic distance (Rogers' 1972 (modified by Wright 1978)) between sites ranged from 0.083 to 0.415 (Table 4.12). The most similar sites were Mt Wellington and Mt Field. Both Gloucester Falls and Wentworth Falls were equally different from the Little Florentine site and these were the most genetically distant pairs of sites. Gloucester Falls and Wentworth Falls are both geographically distant from Little Florentine. However, some sites such as Frodshams and Creepy Crawly, were in very close proximity but also quite genetically distant (Table 4.12). When sites were grouped into localities as indicated in Table 4.4, and the genetic distances were compared (Table 4.13), it was found that the NSW sites were the most genetically distant group from all others and they were on average most closely related to the Victorian sites. This result is not surprising given the morphological divergence of these populations (Schodde 1969) and their geographical remoteness from other, particularly Tasmanian, populations. The Victorian sites however were more similar to the Tasmanian populations than the N.S.W ones. The most similar localities were those sites in the north west and south west. The most genetically distant localities in Tasmania were east central south and the north west followed by the north west and south east (Table 4.13).

At the regional level the mainland populations were distinct from the Tasmanian populations (Table 4.14). Within Tasmania the most differentiation, based on the average genetic distance, was between the sites in the west coast region and the sites in the central south region, however there was almost as much differentiation between the central south sites (Table 4.14). The most similar regions, were the central south and the east coast (Table 4.14).

UPGMA and Distance Wagner tree groupings methods were compared (Figures 4.6 & 4.7). Broadly similar groupings were made by both methods. Both showed a major separation of the N.S.W. sites, and then the Victorian sites from the Tasmanian ones (Figures 4.6 & 4.7). An exception to the separation of Victorian sites was the inclusion of the Donna Buang site amongst the Tasmanian ones (Figures 4.6 & 4.7). This may indicate greater similarity and more recent divergence of the Victorian and Tasmanian populations. Distance Wagner trees have been postulated to be superior to UPGMA trees since they do not assume homogeneity of substitution rates as does the UPGMA (Strauss *et al.* 1992). The optimised distance Wagner tree (Figure 4.7) had the best fit to the data on the basis of several goodness of fit statistics and was used for all further discussion.

Table 4.12 Genetic distances (Roger's 1972 (modified by Wright 1978)) between populations of *A. moshatum* .

SITES	MFS	MFH	MWS	BHS	LFS	CCS	FSS	BIS	MUF	WRS	THS	DBS	TBS	ERS	WFS	GFS	NAS	ARS	MHS	MRS	BTS	LIF
MFS	*****																					
MFH	0.231	*****																				
MWS	0.083	0.232	*****																			
BHS	0.173	0.157	0.148	*****																		
LFS	0.261	0.157	0.242	0.165	*****																	
CCS	0.214	0.259	0.184	0.154	0.245	*****																
FSS	0.200	0.231	0.206	0.141	0.240	0.167	*****															
BIS	0.154	0.206	0.136	0.128	0.169	0.179	0.197	*****														
MUF	0.217	0.257	0.224	0.174	0.288	0.241	0.111	0.246	*****													
WRS	0.205	0.184	0.209	0.103	0.178	0.179	0.113	0.145	0.174	*****												
THS	0.244	0.184	0.227	0.158	0.174	0.254	0.273	0.193	0.286	0.197	*****											
DBS	0.228	0.230	0.246	0.183	0.213	0.208	0.163	0.180	0.233	0.133	0.251	*****										
TBS	0.285	0.284	0.298	0.255	0.303	0.246	0.260	0.282	0.318	0.255	0.300	0.188	*****									
ERS	0.197	0.231	0.224	0.200	0.282	0.204	0.202	0.247	0.250	0.213	0.267	0.204	0.179	*****								
WFS	0.347	0.393	0.363	0.354	0.415	0.340	0.336	0.380	0.369	0.351	0.389	0.281	0.190	0.278	*****							
GFS	0.381	0.395	0.390	0.367	0.415	0.411	0.361	0.401	0.354	0.371	0.395	0.325	0.303	0.364	0 238	*****						
NAS	0.239	0.228	0.240	0.170	0.200	0.237	0.162	0.198	0.181	0.144	0.230	0.190	0.314	0.277	0.371	0.332	*****					
ARS	0.262	0.265	0.282	0.203	0.270	0.235	0.154	0.251	0.177	0.158	0.264	0.185	0.279	0.239	0.346	0.371	0.159	*****				
MHS	0.170	0.229	0.198	0.173	0.256	0.213	0.146	0.186	0.170	0.154	0.257	0.175	0.264	0.207	0.329	0.320	0.134	0.166	*****			
MRS	0.222	0.257	0.242	0.201	0.270	0.250	0.158	0.234	0.157	0.178	0.275	0.210	0.306	0.251	0.355	0.320	0.103	0.145	0.082	*****		
BTS	0.258	0.296	0.285	0.244	0.323	0.286	0.202	0.294	0.182	0.223	0.294	0.249	0.323	0.263	0.346	0 330	0.164	0.131	0.146	0.096	*****	
LIF	0.200	0.214	0.195	0.128	0.220	0.201	0.126	0.194	0.122	0.132	0.214	0.195	0.288	0.231	0.348	0.338	0.108	0.131	0.129	0.107	0.138	*****

Site Codes: MFS- Mt Field; MFH- Mt Field High; MWS- Mt Wellington; BHS- Bun Hill; LFS- Little Florentine; CCS- Creepy Crawly; FSS- Frodshams; BIS- Bruny Is; MUF- Meetus Falls; WRS- Wyefield Riv; THS- Tahune; DBS- Donna Buang; TBS- Tarra Bulga; ERS- Errinundra; WFS- Wentworth Falls; GFS- Gloucester Falls; NAS- Newall Ck; ARS- Anthony Rd; MHS- Murchison Hwy; MRS- Milkshake Res; BTS- Balfour Track; LIF- Liffey Falls

Table 4.13. Genetic distances (Rogers' 1972 (modified by Wright 1978)) between *A.moschatum* populations averaged by regions.

Regions	No.of pops.	Mainland	West Coast	Central South	East Coast
Mainland	5	0.255 (.179- .364)			
West Coast	6	0.285 (.175- .371)	0.129 (.082- .166)		
Central South	5	0.293 (.163- .415)	0.225 (.126- .323)	0.22 (.157- .261)	
East Coast	6	0.294 (.133- .401)	0.206 (.122- .294)	0.191 (.083- .288)	0.183 (.103 - .286)

The Tasmanian populations were broadly divided into two groups, which roughly divided the south and southeastern populations from the western and northern populations (Figures 4.1 & 4.7). However, the three geographically close sites, Little Florentine, Frodshams and Creepy Crawly were split at this level. The grouping of Little Florentine with high Mt Field and Tahune seemed to correspond to their shared low frequencies of PGI - 3 allele A and high frequencies of its allele B which was unlike their proportions in other populations (Table 4.6). The Bun Hill site diverged from other Tasmanian sites fairly early in Figure 4.7. This site is the most south easterly site sampled, it is on a peninsula and geographically isolated from other populations by water and other vegetation types. Therefore it is quite likely to have been isolated long enough for divergences to have occurred due to drift and selection. Most of the divergences among Tasmanian sites are small as can be seen from the tree (Figure 4.7). Therefore more attention should be given to the larger divergences and to variations of allelic composition.

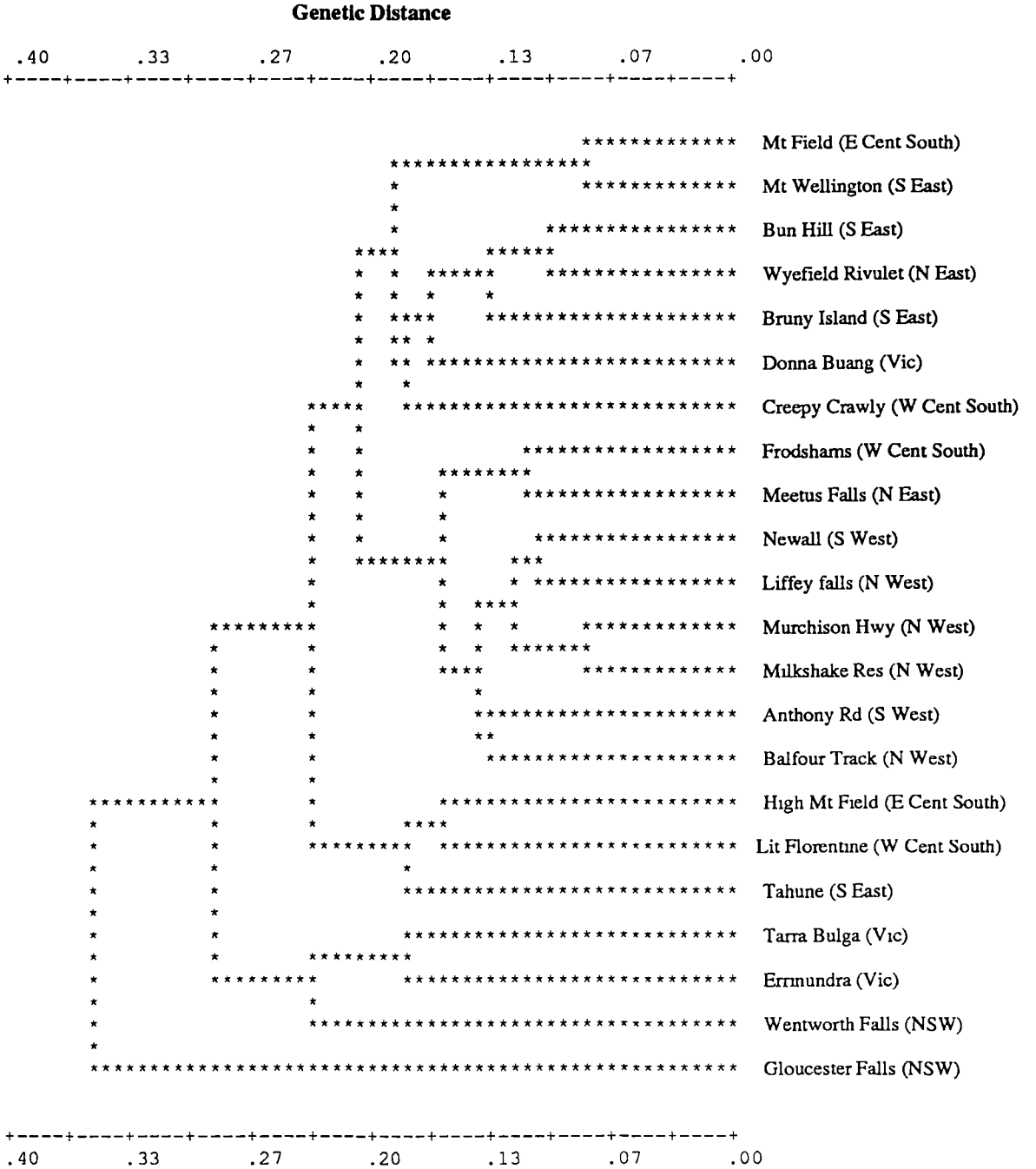
Relationships between Genetics and Site Variables

The results of the Mantels test suggest that genetic distance among sites reflects other ecological or environmental differences between them (Table 4.15). There were significant correlations between three of the environmental/ecological distance measures and genetic distance. Increases in climatic difference, geographic distance and density were all significantly positively correlated with increasing genetic distance (Table 4.15). These positive correlations with genetic distance suggest isolation by distance and differing selection pressures may have contributed to the genetic differentiation observed among sassafras sites.

Table 4. 14. Genetic distances (modified Rogers' 1972 (Wright 1978)) between *A.moschatum* sites averaged by local areas .

Local Area	No.of pops.	Victoria	N.S.W.	South West	North West	Mt Field	Frodshams	North East	South East
Victorian	3	0.19 (.179 - .204)							
N.S.W.	2	0.29 (.190 - .364)	0.238 (.238 - .238)						
South West	3	0.237 (.175 - .314)	0.345 (.320 - .371)	0.153 (.134 - .166)					
North West	3	0.258 (.195 - .323)	0.34 (.320 - .355)	0.127 (.082 - .164)	0.114 (.096 - .138)				
Mt Field	2	0.242 (.197 - .285)	0.379 (.347 - .395)	0.232 (.170 - .265)	0.241 (.200 - .296)	0.231 (.231 - .231)			
Frodshams	3	0.231 (.163 - .303)	0.38 (.336 - .415)	0.208 (.146 - .270)	0.226 (.126 - .323)	0.22 (.157 - .261)	0.217 (.167 - .245)		
North East	2	0.234 (.133 - .318)	0.361 (.351 - .371)	0.164 (.144 - .181)	0.166 (.122 - .223)	0.215 (.184 - .257)	0.185 (.111 - .288)	0.174 (.174 - .174)	
South East	4	0.244 (.180 - .300)	0.38 (.354 - .401)	0.221 (.170 - .282)	0.233 (.128 - .294)	0.179 (.083 - .244)	0.195 (.141 - .273)	0.198 (.103 - .286)	0.165 (.128 - .227)

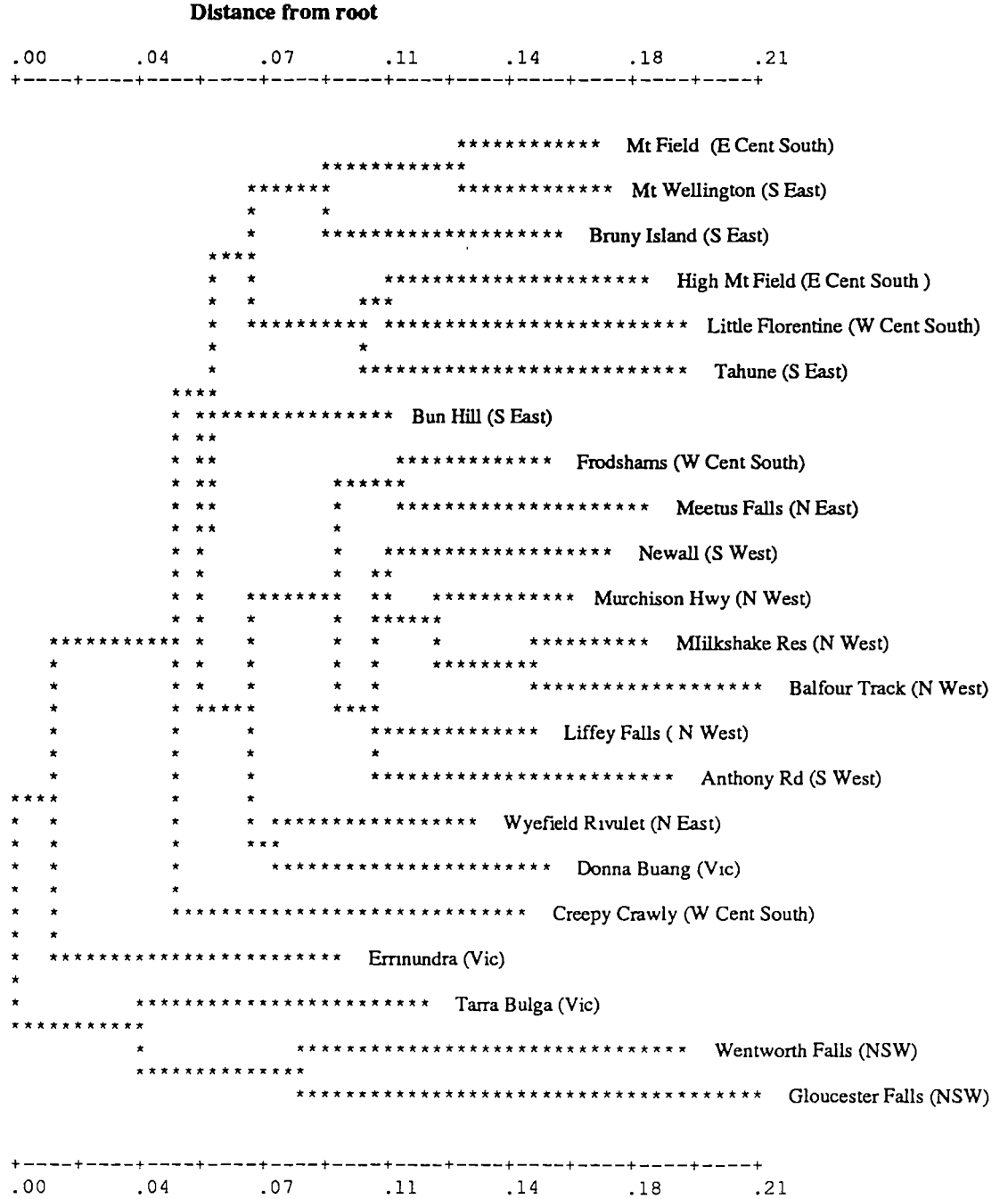
Figure 4.6. UPGMA cluster dendrogram using Rogers' 1972 (modified byWright 1978) genetic distance coefficient, illustrating the genetic relationship between all sites.



Goodness of fit statistics

Farris (1972) "f" = 7.585
Prager and Wilson (1976) "F" = 14.029
Percent standard deviation (Fitch and Margoliash,1967) = 19.904
Cophenetic correlation = .827

Figure 4. 7. Distance Wagner tree illustrating genetic relationships in *A.moschatum* populations,using the modified Rogers' 1972 genetic distance (modified by Wright 1978) and produced after optmisation by rooting at midpoint of the longest path.



Total length of tree = 1.881

Goodness of fit statistics

Farris (1972) "f" = 4.263
Prager and Wilson (1976) "F" = 7.884
Percent standard deviation (Fitch and Margoliash,1967) = 10.937
Cophenetic correlation = .948

Table 4.15. Summary of Mantels G values for tests comparing pairs of distance matrices between *A. moschatum* populations. G = (Z - Expected value)/Standard Error. * P< 0.05, ** Bonferroni significance level (0.05/11) ⇒ P< 0.005.

Matrices	Species	Density	Geography	Genetics
Climate	*-1.64	0.57	**3.02	**2.47
Species		-0.15	0.75	*-1.64
Density			0.88	**4.06
Geography				**4.95

DISCUSSION

There was a variety of size distributions observed among stands of *Atherosperma moschatum* in this study (Figure 4.3 & 4.4). However, few exhibited the reverse J type distributions typical of the continuously regenerating populations (Hett and Loucks 1986) previously reported for this species (Read and Hill 1988). However, all vegetative shoots, up to 5cm diameter, from the base of main stems were included in the analysis of Read and Hill (1988) and may thus reduce the comparability of their results with those of this study. Generally the results suggest that regeneration and stand maintenance in sassafras does not occur by rapidly thinning of large cohorts of mature seedlings, but by fewer juvenile recruits, each with a greater chance of success. These few juvenile recruits, may remain suppressed for long periods before final establishment in the canopy. Such a regeneration mode would be consistent with regeneration through vegetative means, but also with high mortality of very young cotyledon stage seedlings, with the relatively few survivors having a good chance of success (Canham 1989). High mortality of germinants has been observed in sassafras populations (Read 1989, Neyland 1991). It has been postulated that *A. moschatum* replaces itself largely by vegetative means in rainforest (Read and Hill 1988). However, estimations of the amount of vegetative regeneration taking place may have been exaggerated, since it is difficult to establish the origin of a stem once it exceeds a small size. In addition many of the environments where one might find vegetative shoots, such as fallen logs and at the base of mature trees, are also places where seedlings often establish and survive, as they may be protected from predation (Schnabel *et al.* 1991). Vegetative growth may therefore be advantageous, since it avoids the vulnerable seedling stage (Johnson & Lacey 1983). In most populations there were fewer trees in the largest size classes (Figure 4.3), which may suggest self thinning of medium sized trees, since the largest trees were more widely spaced. The size distributions varied between sites (Figure 4.3). There was a fairly even distribution amongst size classes in some populations (e.g. Mt. Wellington). However, other populations had irregular distributions (e.g. Mt. Field), suggesting

that seedling or sapling recruitment may have fluctuated over time. Some stands had almost modulating distributions of sizes (e.g. Errinundra and Tarra-Bulga), suggesting pulses of successful recruitment to the adult population. *A. moschatum* is relatively long lived, therefore few successful recruits are needed to maintain and replace existing populations. While vegetative regeneration is obvious in Tasmanian populations of *A. moschatum*, it becomes less so in Victoria. No evidence of any vegetative sprouting, typical of Tasmanian sassafras was seen in the NSW populations which tended to branch from the main stem rather than send out basal sprouts (confirmed by J.B.Williams and J.Hunter pers comm.).

The size (diameter and height) structure was not related to population size. For example the small isolated eastern Tasmanian stands demonstrated a range of stand structures and regeneration patterns similar to larger western populations. These results suggest therefore that the site conditions and stand histories determine the nature of sassafras stand structure at individual sites, rather than there being generalised patterns for the species.

Many *A.moschatum* stands do not conform to Hardy-Weinberg expectations and are inbred (Table 4.8). The degree of inbreeding varied among populations (Table 4.9). Inbreeding could be due to many factors, including selfing as these trees are insect pollinated. Inbreeding has generally been observed to be high in insect pollinated plants, since pollinators frequently only travel short distances between visits (Loveless and Hamrick 1984). The level of inbreeding has often been associated with the density of populations (Antonovics and Levin 1980). In denser stands mate selection is more likely to be non-random, as pollination is more likely to arise from close neighbours which may already be in family groupings (Antonovics and Levin 1980). Sparser stands encourage longer distance within stand pollination which is therefore more likely to be random and occur between unrelated individuals (Shaw and Allard 1979). In insect pollinated plants the nature of the pollinator will influence pollination success in less dense populations (Shaw and Allard 1979). In this study, however, there was no consistent pattern relating density of stands with inbreeding, although there was a relationship between density and genetic similarity (Table 4.15).

Even if sassafras is mostly outcrossing, effectively inbreeding populations can arise if seed dispersal is poor, or if most seeds are dropped close to the parent tree (Shaw and Allard 1979). If this seed is successful clusters of genetically related family groups would arise (Epperson 1989, Dewey and Heywood 1988). Groups of genetically related trees could arise in this manner, especially if the parent tree

provides 'safe' sites or moist microsites which facilitate seedling survival. In some species the offspring germinating under parental trees are selected against, reducing this effect (Shaw and Allard 1979). Even though sassafras seeds can disperse great distances (Neyland 1991), like most species this distribution is leptokurtic and the majority of seed falls beneath the parent tree. This may be especially so in closed rainforest (Read and Hill 1988). A similar situation can also arise if trees are reproducing vegetatively, whereby clumps of clones are produced. Since spatially closer trees are more likely to pollinate neighbour trees regardless of pollination method, unless self or like pollen is selected against, pollination within family clusters will lead to further inbreeding (Jain 1976). Therefore vegetative reproduction and seed dispersal may have a major impact on inbreeding in *A. moschatum* populations.

Effective inbreeding can also arise as a result of low genetic diversity in a population, which may be maintained by isolation from other populations. In the Tasmanian populations sampled, there was no consistent difference in the amount of inbreeding observed in small isolated populations compared with stands from larger assemblages. In NSW populations the cause of inbreeding is unlikely to be due to vegetative regeneration, as none was observed in these sites, therefore more likely due to the result of the development of localised clusters of related trees, or of limited gene flow between populations.

It is clear that these mechanisms are also likely to lead to spatial heterogeneity or structure within *A. moschatum* populations, which may also lead to localised differentiation of genotype frequencies by allowing random drift in genetic composition, depending on the local genetic composition (Turner *et al.* 1982). Differentiation within larger populations was observed among the Creepy Crawly and Frodshams sites, which were approximately 1.5 kilometres apart and located within a large semicontinuous population. The altitude and climatic conditions of each site were approximately the same. However, the species composition and structure of each site was different (Figures 4.3 & 4.5). The Creepy Crawly site tended towards a thamnic community type (Jarman *et al.*1984) and was more species rich than the callidendrous Frodsham site. Thus there was some evidence of environmental differences between the sites, possibly relating to soil fertility (Jarman *et al.*1991). The sites were quite genetically distinct (Table 4.12) with markedly different allelic diversity and composition for three enzymes systems (Table 4.6). Poor gene flow has enabled differences resulting from differential selection or chance drift in the two sites to be maintained. The other two closely spaced sites, Mt Field and High Mt Field, were also genetically distinct (Tables 4/6 & 4.12), again

suggesting limited gene flow. However, in this case it is reasonable to assume that the high site would be subjected to strong selection pressures, since it is near the altitudinal limits of the species at this site and that this may overcome local gene flow between these two sites. These results indicate that differentiation can take place over relatively short distances in *A. moschatum*.. Genetic differentiation among sites was correlated with environmental/ecological differentiation among sites (Table 4.15), suggesting that selection may have contributed to genetic differentiation among sites in this species.

Although there were differences between sites, particularly at the broadest geographical scale, most of the variation in *A. moschatum* was recorded within rather than between sites (Tables 4.10 & 4.11). This result is consistent with what would have been predicted based on the results of the extensive survey of genetic diversity in plants by Hamrick and Godt (1989). The genetic variation within Tasmanian *A. moschatum* sites was variable, but greatest in the Murchison Highway site, which contained all the rare or uncommon alleles (Table 4.6). Otherwise these alleles were distributed throughout the range of the species in Tasmania. The genetic diversity within sites of *A. moschatum* was fairly high overall compared to other plant species (Hamrick and Godt 1989) (Table 4.7). It was similar generally to those found in long lived, late successional, well dispersed, outcrossed species (Hamrick *et al.* 1979). Populations of late successional species such as *A. moschatum* are usually more stable and long lived species may have greater within population variability since many generations may be present at once (Loveless and Hamrick 1984). Sassafras had much higher proportions of polymorphic loci than the average reported for species which reproduce both sexually and asexually (Hamrick and Godt 1989).

In *A. moschatum* the average among site genetic diversity (GST or FST of 0.178) (Table 4.10) was lower than the average for dicotyledonous (0.273) or temperate species (0.246) generally (Hamrick and Godt 1989). This result is however, higher than the average values reported for other long lived (0.076), late successional (0.101) or wind dispersed (0.143) species (Hamrick and Godt 1989). It has been argued (Loveless and Hamrick 1984) that there are greater chances of gene flow between populations of long lived species due to the extended time scale involved, which will counteract the effects of drift. It has been said (Ellstrand 1992) that only a few migration events are needed to restore diversity in a population that is inbred and reduce the effects of drift. As mentioned above, insect pollinated species generally have decreased gene flow between populations, effectively increasing diversity among populations (Loveless and Hamrick 1984). However, *A. moschatum*

has lower between population diversity than the average reported for other insect pollinated species (Loveless and Hamrick 1984). Synchronous sexual reproduction which occurs in *A. moschatum*, will tend to increase diversity generally by promoting the mixing of genes. Monoecious and dioecious species generally have lower among population diversity than that found in *A. moschatum* (Loveless and Hamrick 1984). However, this may be because most of the species studied in this category, were wind pollinated gymnosperms which generally have greater gene flow and therefore lower diversity among populations (Loveless and Hamrick 1984). Seed adapted for wind dispersal, generally allows greater migration between populations and so tends to reduce among population differentiation (Hamrick and Godt 1989). The mainland sites were generally more diverse than the Tasmanian sites and would be expected to have less seed dispersal among sites. Their differentiation from Tasmanian sites (Table 4.13) increased the overall diversity observed among *A. moschatum* sites.

The New South Wales stands sampled were shown to be considerably divergent in their genetic composition from the rest of the *A. moschatum* stands (Table 4.6 and Figure 4.7). The morphology of *A. moschatum* plants at these sites was also very distinctive and considerably different from all southern sites (Figure 4.8). Combined these attributes suggest that the New South Wales populations warrant taxonomic revision as they may have become a different species, and at least should be regarded as a distinct subspecies, as has been suggested by others (Schodde 1969). As *A. moschatum* is currently regarded as a monophyletic genus the distinct nature of the New South Wales populations is significant. The New South Wales stands were clearly most closely related to the Victorian stands, since they shared several allelic variants absent or rare in Tasmanian stands. There was however considerable diversity between the Victorian stands, probably reflecting their location in three distinct and isolated regions, containing rainforest and mixed forest in Victoria (Figure 4.1). The Victorian stands however were structurally, environmentally and morphologically more similar to Tasmanian stands, and while genetically distinct, showed more affiliation with Tasmanian than New South Wales stands (Figure 4.7). Within Tasmania there was some divergence of the southeastern sites from the northern and western sites, due to the distribution of some allelic variants and the proportions of particular alleles (Table 4.6 and Figures 4.6 & 4.7). This result is not surprising as it may reflect two major sources of origins for post glacial populations. Refugia in the south east would have been isolated from western populations during the Last Glacial (Kirkpatrick 1986) and so populations arising from each area may be expected to reflect this.

Figure 4.8 Contrast between the morphological form of A. moschatum from Gloucester Tops (N.S.W.) below, and a typical Tasmania form above. Both specimens have been grown in a bush house for several years in Hobart Tasmania.

The results show Tasmanian small populations to be indistinguishable from larger ones, in terms of their variability, breeding, genetic diversity, density and stand structure. There was no evidence of principally clonal populations. The findings suggest that sexual as well as vegetative reproduction, has contributed to stand development, and that gene flow has been relatively important in these populations. Schwaegerle & Schaal (1979) suggested that only a few migrants are needed to maintain diversity. However, migrants will have more significance in small populations than larger ones where they will tend to have less relative importance (Ellstrand 1992). These results seem more reasonable because the vegetative capacity and longevity of *A. moschatum* enables it to persist for long periods, increasing the likelihood of otherwise possibly rare dispersal events occurring within a tree's lifespan, and reducing the frequency required for successful seedling establishment

Coates (1988) found no relationship between population size and genetic diversity in *Acacia anomala*, but noted that the small populations exhibited unexpectedly high levels of genetic diversity. Coates (1988) suggested in his study of *Acacia anomala,* that the greatest genetic diversity existed within the most inbred populations, in contradiction to the findings of the survey by Hamrick *et al.* (1979). Since populations of all sizes were inbred in the study, and there were no apparent trends, this does not appear to have been a major determinant of genetic diversity in *A. moschatum* populations, although many of the small isolated populations, e.g. in N.S.W. and Victoria, contained more genetic variability than the larger populations.

It may be speculated that sassafras populations underwent size reductions or bottlenecks to varying degrees during the Last Glacial. There may have been some scattered populations from which new populations could arise and from which stocks could be replenished. In such situations the potential of wind dispersal of *A. moschatum* seeds would have been more important than perhaps it has been recently. Theories based on work done on species with short generation times (Lande 1988) predict 400 generations are required to fully overcome the effect of a bottleneck. There may have been as few as 40 and unlikely to be more than 100 generations passed in *A. moschatum* populations since the last glacial cycle. Lande's (1988) hypothesis therefore suggests that *A. moschatum* populations may still be affected by such population reductions and founder effects and not be expected to have reached equilibrium (Whitlock and MacCauley 1990). However, the question arises as to whether long lived species will ever reach genetic equilibrium due to the fast pace of other environmental changes, especially at the hands of humans.

The NSW populations have probably been isolated a very long time, and there is both genetic and morphological evidence of their divergence (Figures 4.7 & 4.8). Their differentiation is likely to have been driven by selection and maintained by isolation. These populations should be considered to be rare and distinct forms of this species and therefore given high conservation status. The mainland populations diverge as they increase their distance from Tasmania, but all are genetically distinct from Tasmanian ones, reflecting their isolation. In order to maintain the genetic diversity of this species some populations throughout its distribution should be conserved.

Since current levels of genetic variability do not seem to be adversely affected by the population size, and since populations can persist for at least several hundreds of years, it would seem that the short term genetic viability of populations is not determined by their size. However, it may be that for long term survival there needs to be a network of such small populations within dispersal distance to help maintain diversity within stands and to replenish or replace populations which do have their populations reduced.

CHAPTER 5 :

The spatial genetic structure of *Atherosperma moschatum* stands.

INTRODUCTION

The spatial distribution of genetic variability within natural plant populations may significantly influence evolutionary and ecological processes (Epperson 1989, Lewontin 1974, Endler 1977, Wright 1978, Brown 1978). The spatial distribution of genetic variation in populations forms an integral part of neighbourhood concepts (Wright 1978), as well as theories of allopatric and parapatric speciation (Mayr 1970). Even when the scale of structure is fine, localised genotypic combinations may arise, and small demes may become differentiated, despite being part of a large ensemble (Wright 1978). Survival may depend on tolerance of locally patchy environmental factors such as soil type (Bradshaw 1984). Spatial structure of genetic variation within populations has immediate ecological-genetic consequences for plants, since it may result in aggregates of particular genotypes.

Knowledge of population spatial structure is important when selecting natural populations for conservation, or sampling them for breeding programs. It should be taken into account in order to maximise diversity, and not to misrepresent species or population diversity (Epperson 1989). If the presence of spatial structure is ignored, it may lead to misinterpretations of the significance of breeding systems or the selective significance of particular genotypes (Epperson 1989). Genotypic structuring over short distances within populations may be caused by limited dispersal of pollen or seed, spread of vegetative clones or by selection within a patchy environment (Epperson 1989, Dewey and Heywood 1988).

Spatial autocorrelation analysis has been used to study genetic structure within plant populations (Dewey and Heywood 1988, Coates 1992, Epperson and Clegg 1986, Waser 1987, Fortin *et al.* 1989, Argyres and Schmitt 1991, Schnabel *et al.* 1991, Knowles *et al.* 1992, Schoen and Latta 1989). Spatial autocorrelation analysis makes no assumptions about the scale of the structure (Dewey and Heywood 1988), and since individuals can be used for the analysis, any scale of pattern can be analysed. The results of studies to date have revealed localised structure over short distances in some species' populations (Wagner *et al.* 1991, Schoen and Latta 1989, Fortin *et al.* 1989, Perry and Knowles 1991, Argyres and Schmitt 1991, Schnabel *et al.* 1991, Epperson and Clegg 1986). However, random genetic distributions have been found

in other species (Coates 1992, Epperson and Allard 1989, Dewey and Heywood 1988, Waser 1987). Differences in life histories, dispersal characteristics, breeding systems and stand histories, may account for some of the differences in results between species and populations (Xie and Knowles 1991, Coates 1992, Schoen and Latta 1989). Fine scale substructuring seems to be the most common kind of genetic structure within populations (Epperson 1989). However, in some species and populations gene flow appears to be greater than expected from their biology and environment, leading to randomisation of their genetic structure (Waser 1987).

The results from the previous chapter and previous studies suggest that *A. moschatum* stands are likely to exhibit genetic substructuring. For example, *A. moschatum* is likely to have restricted pollen dispersal since it is insect pollinated, and though capable of long distance dispersal, most seed in closed canopy situations falls beneath the parent tree (Read and Hill 1988). F statistics (Wright 1965) based on isozyme variation indicated that most variation was within populations and that they were mostly inbred and not in Hardy-Weinberg equilibrium (Chapter 4). It was postulated that inbreeding could be the result of two factors: vegetative reproduction; and the development of family clusters due to poor pollen and seed dispersal. Therefore this study used spatial autocorrelation analysis to investigate the spatial distribution of allozyme genotypes at six enzyme loci, within seventeen natural stands of *A. moschatum.*

METHODS

Spatial autocorrelation analysis tests whether the observed value of a variable at one point or locality is independent of values of the variable at neighbouring points or localities. If dependence exists the variable is said to exhibit spatial autocorrelation (Sokal and Oden 1978a). For the purposes of this type of analysis the distribution of points is considered as given (Legendre and Fortin 1989, Sokal and Oden 1978a). Spatial autocorrelation of trees within stands was calculated separately for six enzyme loci in each of seventeen sites and the results compared. The genetic analysis and stand structure of these sites is given in the previous chapter.

The actual co-ordinates of all *A. moschatum* trees within the sample sites were used for the analysis. These formed an irregular lattice of sample points. Pairs of trees were considered to be 'neighbours' within a radius of ten metres. Ten metres was estimated as the approximate area of influence of an average *A. moschatum* tree based on field observations. This distance also took into account the density of trees (Table 5.1), so that there would be enough 'neighbours' to give statistically

meaningful results. The variation in autocorrelation and hence genetic relationships between trees that occurs with increasing distances between trees was analysed by assessing autocorrelation with increasing distance classes. These had an annulus increase of ten metres.

Pairs of 'neighbour ' trees were given a binary score of 1, if 'joined', by the pair definition, or 0, if not 'joined' within each distance class. Thus all pairs of genotypes were assessed for the number of times they co-occurred within a distance class. This number was compared to the number of joins expected and the variance for each type of join, if tree genotypes were randomly distributed (assuming sampling without replacement) (Sokal and Oden 1978a). If there were significant excesses of 'joins' between genotype pairs, those pairs of genotypes would be said to be positively autocorrelated. A deficiency of joins would indicate negative autocorrelation (Epperson and Clegg 1986).

In the case of nominal data such as this, the significance is assessed by the estimation of the Standard Normal Deviate (SND), which is compared to a T distribution (Sokal and Oden 1978a). Corrections for small sample sizes and small degrees of freedom are given by Sokal and Oden (1978a). Generally SND = (observed - expected)/ $\sqrt{}$variance, and the degrees of freedom are given by 2(expected)^2/$\sqrt{}$variance (Sokal and Oden 1978a). The observed and expected join counts and the variances were calculated using a FORTRAN program (written by M. Zaluki) based on the formulae given by Sokal and Oden (1978a).

Correlograms were used to investigate the patterns of association of genotypes with increasing distance at each site. A correlogram is a plot of the standard normal deviate (SND) of each pair type at each increasing distance class or annuli (Legendre and Fortin 1989). Increments of ten metres were used in this analysis. To assist with interpretation of results the SND values were also averaged for all like genotype pairs or all unlike genotype pairs at each site and these average SND values were plotted. All enzymes at each site were then compared on the same graph for these broad genotype pair groupings. The significance of correlograms was checked using the Bonferroni method of correcting for multiple tests (Oden 1984), such that at least one SND value is significant by this method (Legendre and Fortin 1989) where $\chi' = \chi/\upsilon$ (χ = 0.05 sig level; υ = number of tests). Since some values in all correlograms were significant at Bonferroni levels the Correlograms were generally considered significant (Legendre and Fortin 1989). However, the power and significance at each distance class will be different due to the varying number of connected pairs at each distance. Therefore correlograms are most powerful at the shortest distance classes

(Epperson 1989). In this study patterns at distances greater than thirty metres are unlikely to be reliable due to the edge effects of the transects (Epperson 1989).

Scatter plots mapping the location, diameter and genotypes of each tree were used as an aid in interpretation of the spatial autocorrelation results.

RESULTS

The results from the different enzymes were consistent at each site as well as between sites, particularly at short distances (ten metres). Generally pairs of trees of the same genotype (like trees) were significantly positively autocorrelated at short distances (up to ten metres). A comparison of all like pair correlations in all enzymes found that, overall 73 % were significantly positively autocorrelated at ten metres with ranges from 67% to 82% between enzyme systems (Table 5.2). The remainder gave nonsignificant results (Table 5.2).

Table 5.1. Summary of the sample size and average distance between trees at each sassafras site.

Site	Sample Size	Ave Distance Between Trees
Mt. Field	40	3.2
Mt. Wellington	55	4.7
Bun Hill	62	3.5
Little Florentine	25	4.2
Creepy Crawly	60	4.4
Frodshams	44	2.2
Bruny Island	25	4.6
Meetus Falls	45	3.2
Wyefield Rivulet	61	1.9
Tahune	42	5.3
Newall Creek	46	3.5
Anthony Road	34	3.6
Murchison Highway	55	1.8
Milkshake Reserve	51	3.8
Liffey Falls	60	5.9
Errinundra	30	3.1
Tarra-Bulga	28	7.3

Table 5.2. Summary of spatial autocorrelation results over all sites studied . The summation of pairwise associations between trees of different genotype combinations within a ten metre radius is given. The results of each genotypic pair combination are pooled over all study sites and given as a percentage of the total number of each type of pair combination.

Enzyme	Pair Type	% Sig +ve	% Non Sig	% Sig -ve
PER	Total Like	67	33	
	Homozygote like	76	23	
	Heterozygote like	47	53	
	Total Unlike		23	77
PGI-2	Total Like	82	17	
	Homozygote like	92	8	
	Heterozygote like	61	39	
	Total Unlike		20	80
PGI-3	Total Like	72	28	
	Homozygote like	82	18	
	Heterozygote like	47	53	
	Total Unlike		31	69
SDH	Total Like	74	26	
	Homozygote like	78	22	
	Heterozygote like	60	40	
	Total Unlike	1	37	62
GDH	Total Like	75	25	
	Homozygote like	85	15	
	Heterozygote like	53	47	
	Total Unlike		20	80
AAT	Total Like	70	30	
	Homozygote like	85	15	
	Heterozygote like	46	54	
	Total Unlike		35	65
Mean	Total Like	73	27	
	Homozygote like	83	17	
	Heterozygote like	52	48	
	Total Unlike		28	72

There were stronger associations overall between like homozygous trees than like heterozygous trees. An average of 83% of all homozygous like tree pairs were significantly positively autocorrelated at ten metres. This value ranged between 76% and 92% for different enzymes. Like heterozygote tree pairs were on average significantly positively associated in 52% of cases with a range from 47% to 71% for different enzymes (Table 5.2). By twenty metres the results between pairs of like trees were nonsignificant in most cases, 78% overall (Table 5.3).

Unlike pairs of trees were generally significantly negatively autocorrelated at both ten (72%) and twenty metre (84%) distance classes or else were not significantly autocorrelated (Tables 5.2 and 5.3).

Table 5 .3 Summary of associations, tested using spatial autocorrelation, between tree genotype pairs over all sites within a ten to twenty metre radius. The results of each genotypic pair combination are pooled over all study sites and given as a percentage of the total number of each type of pair combination.

Enzyme	Pair Type	% Sig +ve	% Non Sig	%Sig -ve
PER	Total Like	18	82	
	Total Unlike		22	78
PGI -2	Total Like	21	79	
	Total Unlike		5	95
PGI -3	Total Like	31	69	
	Total Unlike		25	75
SDH	Total Like	23	77	
	Total Unlike		16	84
GDH	Total Like	27	73	
	Total Unlike		3	97
AAT	Total Like	8	92	
	Total Unlike		25	75
Mean	Total Like	22	78	
	Total Unlike		16	84

Correlograms were used to visualise the patterns of associations with increasing distances between trees. The resulting patterns were consistent for either like or unlike genotype pair combinations, as can be seen in the example at Newall Creek (Figure 5.1). Therefore the significant normal deviate (SND) was averaged between all like or unlike pairs for each enzyme. Correlograms for like and unlike pairs for each enzyme were plotted together for each site. From these correlograms two consistent patterns of results emerged. Half the sites (8/17) displayed a pattern of significant positive associations at ten metres, crossing the X axis (SND = 0) at around twenty metres, then becoming significantly negatively autocorrelated. Unlike pair combinations were negatively autocorrelated at ten metres and continued to be significantly negatively associated at greater distances (e.g. Figure 5.2).

The second pattern, observed in seven sites, again consisted of significant positive associations at ten metres for like genotypes. Like autocorrelations dipped at twenty metres, but continued to be positive with greater distance, though mostly nonsignificant (e.g. Figure 5.3). Unlike pairs were initially significantly negatively autocorrelated, but approached zero by thirty metres (e.g. Figure 5.3).

Figure 5.1 Correlogram showing the change in spatial autocorrelation with increasing distance between pairs of trees with like or different GDH genotypes.

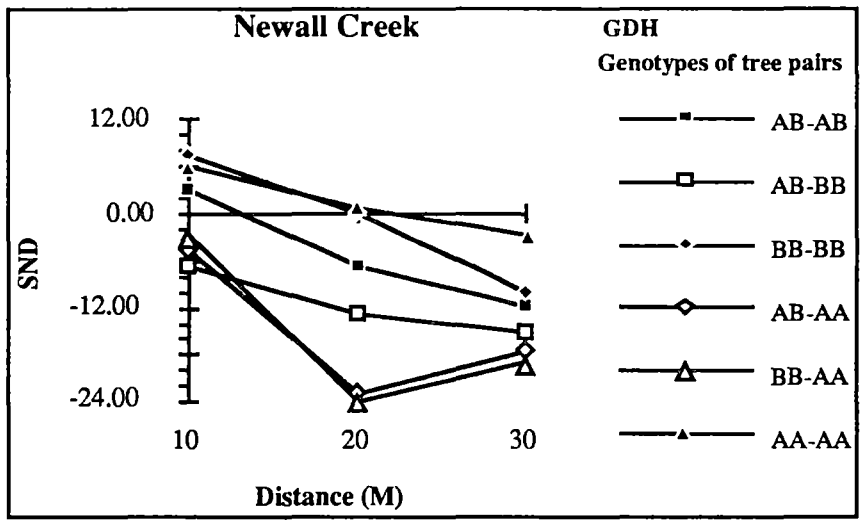


Figure 5. 2 Correlogram showing the relationships between pairs of trees of either like or unlike genotypes with increasing distance apart at Tarra Bulga. Like genotype pair and unlike genotype pair significant normal deviate scores, were averaged for each enzyme loci and used for this plot.

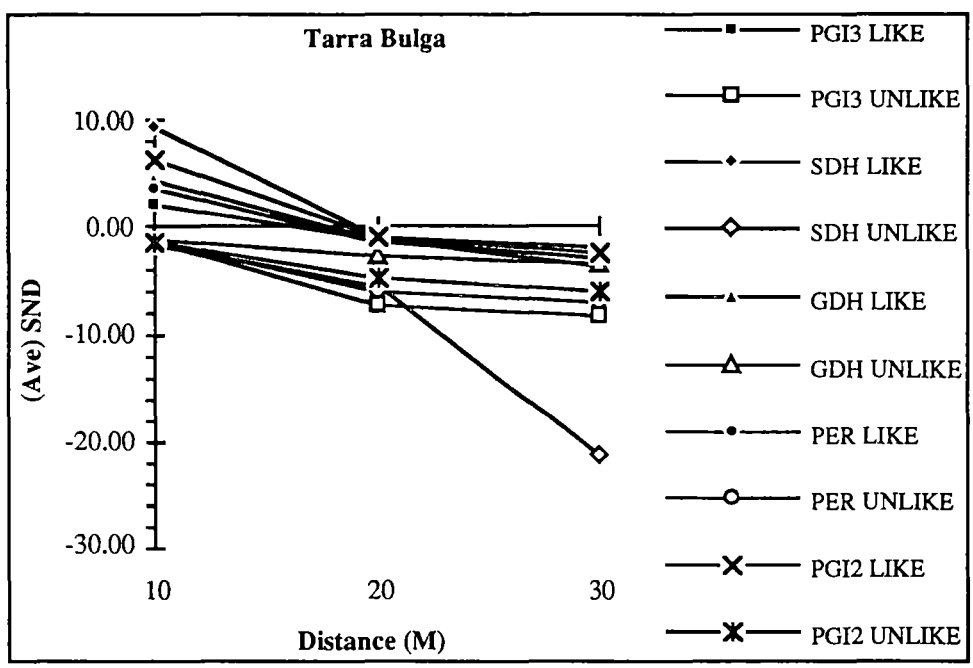
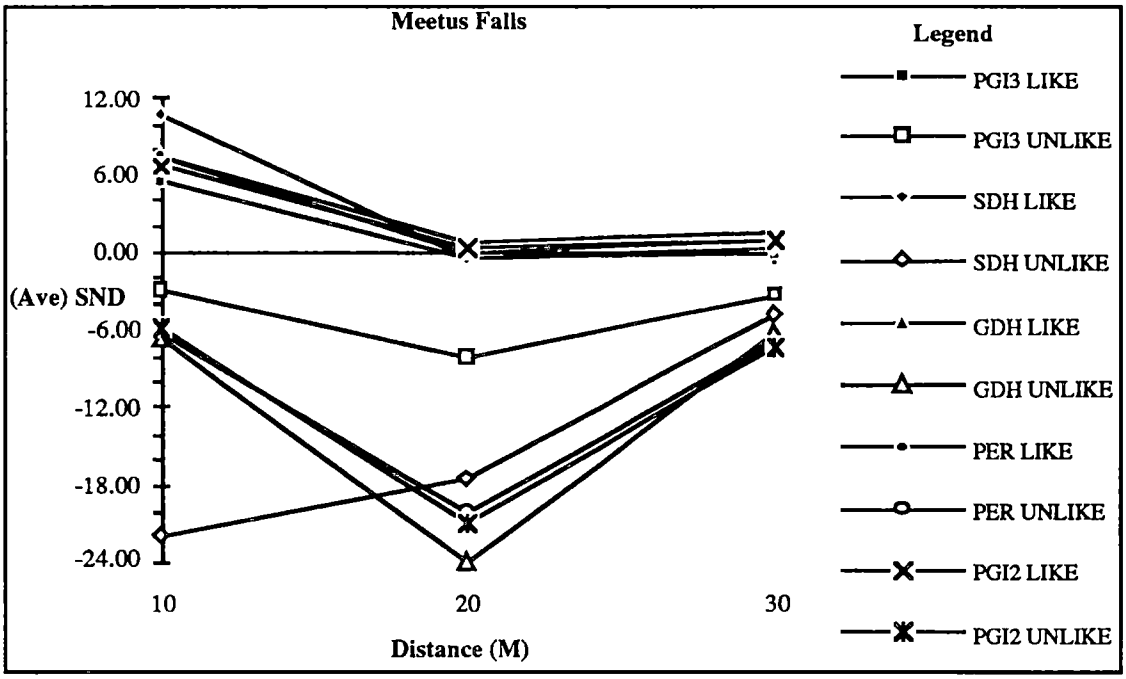


Figure 5. 3. Correlogram showing the relationships between pairs of trees of either like or unlike genotypes with increasing distance apart at Meetus Falls. Like genotype pair and unlike genotype pair, significant normal deviate scores were averaged for each enzyme loci and used for this plot.



Positive autocorrelation at short distances represents clusters of trees with like genotypes (Legendre and Fortin 1989). Negative autocorrelation for short distances can reflect either an avoidance phenomenon such as found among regularly spaced plants, or the sampling interval may be too large to detect associations (Legendre and Fortin 1989).

The first correlogram pattern described above, is the most common autocorrelation pattern found in plants (Epperson 1989, Legendre and Fortin 1989). It is typical of a pattern found in populations with relatively greater proportions of homozygotes and poor dispersal (Epperson 1989). The pattern usually represents aggregates of homozygotes, with zones of heterozygotes intergrading between them, so that unlike trees are negatively associated, especially unlike homozygotes, as they do not occur together (Epperson 1989). The increasing negativity with distance reflects the reduced likelihood of encountering the same genotype with increasing distance (Epperson 1989).

The point at which a correlogram crosses the X axis (SND = 0), or switches sign, is an operational estimate of the length of one side of the 'average' patch. In irregular shaped or sized patches, it equals the average length of the shortest side (Epperson and Clegg 1986). Patch dimensions are primarily determined by breeding structure and dispersal (Epperson and Clegg 1986), which may be affected by density (Antonovics and Levin 1980).

The second pattern observed in the correlograms would be consistent with short distance clustering of like genotypes in an otherwise random spatial arrangement. Positive associations at larger distances are unusual (Epperson 1989), and usually associated with repeated or regular patterns in the environment (Legendre and Fortin 1989). They represent the distance between aggregates of the same genotype.

Over all populations studied there was a high level of consistency for the average patch length (approximately twenty metres). One site, Liffey Falls, had a slightly larger patch length of approximately twenty- five metres.

Figure 5. 4 Correlogram showing the relationships between pairs of trees of either like or unlike genotypes with increasing distance apart at Milkshake Reserve. Like genotype pair and unlike genotype pair, significant normal deviate scores were averaged for each enzyme loci and used for this plot. Like genotype tree pairs remained significantly autocorrelated over the tree distances measured. The dotted lines on the graph indicate the 0.05 significance level for SND values.

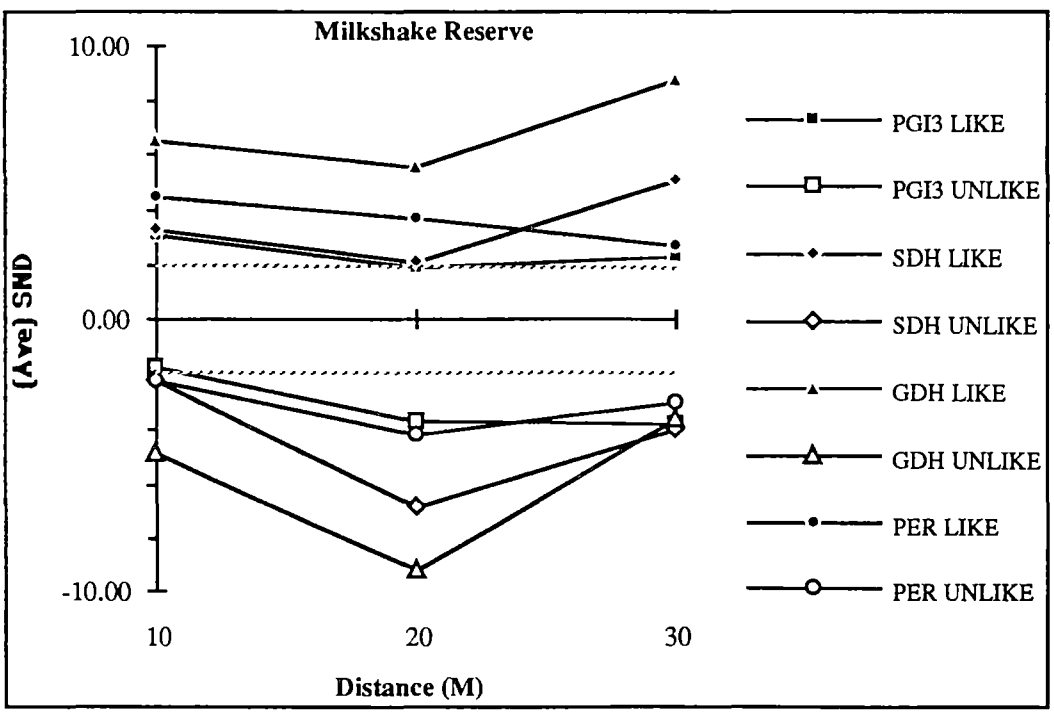
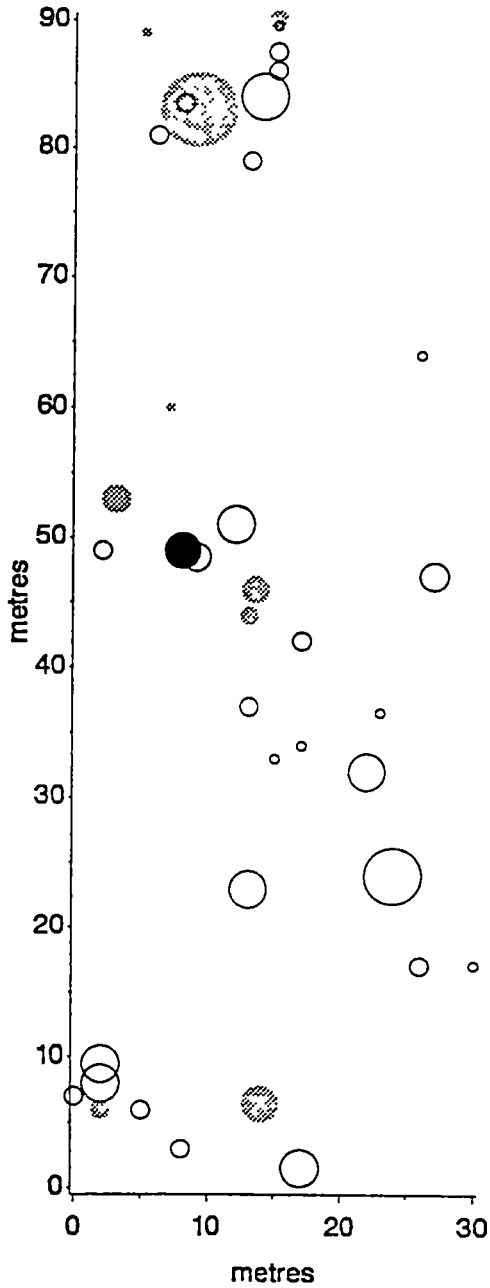


Figure 5.5 Map of the transect of *Atherosperma moschatum* trees at Tahune showing the distribution of GDH genotypes amongst the trees. The tree symbols used are proportional to eight times the actual diameters. (Genotypes; ○ BB, ◍ AA, ● AB)



Two sites Milkshake Reserve and Wyefield Rivulet (Figure 5.4), deviated from all others in their correlogram patterns, in that like trees continued to be significantly positively autocorrelated at all three distance classes. This is an unusual pattern and is most easily explained by the existence of smaller patches which are repeated within the distance classes scored. At these sites like heterozygous genotypes were strongly positively correlated which may suggest small clumps of vegetative clones. Distances between such clumps would be of the order of ten metres. At these sites the average distances between trees was 3.8 metres and 1.9 metres (Table 5.1). There was no apparent association between stand density or stand size structure with the size of the patches measured at each site.

DISCUSSION

The results of this study are consistent with the expectations for this species based on the stand structure and genetic results of the previous chapter (Epperson 1989, Sokal and Wartenberg 1983). Positive autocorrelation of plants with the same genotype at short distances is predicted where reproduction occurs vegetatively (Sokal and Oden 1978b, Oden 1984). In such cases clusters of clones would result. The distribution of genotypes among such clusters however could be random. Heterozygotes would be fewer, but heterozygote and homozygote clusters would not be expected to be structured differently, unless heterozygotes did not reproduce vegetatively.

Short distance positive autocorrelation is also found where family clusters have developed (Epperson 1989, Legendre and Fortin 1989). Modelling studies have shown that in populations with limited gene flow, such as in insect pollinated species or where most seed falls beneath the parent tree, family aggregates quickly develop (Ennos and Clegg 1982). This leads to clusters of homozygotes separated by heterozygous intermediate zones (Epperson 1989, Turner *et al.* 1982). It has also been shown that uncommon long distance dispersal events have little effect on this family structure development (Schnable *et al.* 1991, Epperson 1989). Some weak positive autocorrelation of heterozygotes may result in the intermediate zones of heterozygotes (Epperson 1989).

Both of these two mechanisms for producing small scale genetic structure described above (i.e. limited seed and pollen dispersal), are likely to occur in *A. moschatum* stands. Therefore either or both may be responsible for the substructuring observed in these stands. In the field it is often difficult to distinguish between stems that have arisen vegetatively from those which have arisen from seedlings. This is exacerbated by the fact that many of the sites likely to produce vegetative shoots are also the

places most likely to provide 'safe' germination sites. Similar observations have been made for other species (e.g. Schnabel *et al.* 1991).

Like heterozygote genotype trees were not significantly clumped as often as like homozygote trees in the shortest distance class (0-10m) (Table 5.2). However, since heterozygotes were generally in low proportions in stands (Chapter 4), there may not have been enough like heterozygote pairs to produce a significant autocorrelation. Where significant positive autocorrelation was found, it may have resulted from vegetative reproduction enlarging a zone of heterozygotes between two homozygous family clusters.

The size of patches (~20 m diameter) as determined by the correlograms (Figures 5.1, 5.2 and 5.3) was generally consistent between sites and seems larger than would be expected if such structure was solely due to vegetative reproduction. When genotypic site maps were studied, it was found that dense clumps of stems were often of mixed genotypes, but that scattered trees were often grouped by genotype (e.g. Figure 5.5). Similar results were found by Schnabel *et al.* (1991) and they support the hypothesis that clumps of stems are as likely to be produced by seedling germination as by vegetative growth. Since the size of patches was generally consistent between sites it is unlikely to be generated by spatially varying selection. There was no consistent observable microhabitat heterogeneity within sites to have caused such small scale selection. Other authors have interpreted such consistency of localised structure as generated by short distance gene dispersal (Epperson and Clegg 1986, Schnabel *et al.* 1991, Schoen and Latta 1989).

The sites that showed a pattern of short distance clumping and then nonsignificant or random dispersion of genotypes, were all smaller transects. It appears, from site maps, that one or two major clumps are found in these samples. The rest of the trees may represent the edges of several other patches, and so the results are effectively random outside one patch. Where significant positive correlation is found at greater distances, this will be where another clump of the same genotype is encountered and may also indicate smaller patch sizes in that site (Legendre and Fortin 1989). Where stands are dominated by only a few genotypes the likelihood of this occurring is increased .

There was no difference between genotypes in patch size. Therefore there was no strong evidence for selection of one particular genotype over others within the sites. There was no evidence of differential selection between the different enzymes studied, since the results were consistent for all enzymes. This suggests that the

proportions of alleles or genotypes present at a site may be largely due to chance or historical factors, and the enzymes studied are effectively neutral (Epperson 1989, Schoen and Latta 1989, Schnabel *et al.* 1991).This is consistent with findings from other studies (Epperson 1989, Knowles *et al.* 1992), however in some cases structure was apparent for only some of the enzymes studied (Schnabel *et al.* 1991, Dewey and Heywood 1988).

Despite a diversity of population sizes, degree of isolation, density, stand size structure and stand histories (Chapter 4), there was a high degree of consistency of presence and scale of genetic structure among the stands of *A. moschatum* studied. These results are consistent with hypotheses that plant populations are subdivided into local demes or 'neighbourhoods' of interbreeding, related individuals (Bradshaw 1972, Levin and Kerster 1974). However, the scale of structure here is fine relative to the size of trees and therefore indicating small neighbourhoods. These family groups may be randomly dispersed through the population in this case. This sort of small scale structure has been found in several other studies (Schnabel *et al.* 1991, Perry and Knowles 1991, Epperson and Clegg 1986). It is difficult to compare the scale of pattern between studies since one would expect the scale of pattern to be relevant to plant size. However, in other studies of trees, patch sizes of similar dimensions have been reported (Perry and Knowles 1991, Schnabel *et al.* 1991). Studies in which plants are sampled on a regular grid (eg Epperson and Allard 1989), may miss such fine scale patterns if the sampling step is too large. Fortin *et al.* (1989) demonstrated that irregular sampling is more efficient for pattern detection than a regular lattice, since a range of neighbour distances is included.

The results of a study of *Acer saccharum* stands of mixed age class trees by Perry and Knowles (1991) were similar to this study. They suggested that while *Acer saccharum* seed can be dispersed great distances, seed dispersal in dense forests is restricted. A similar scenario is suggested for *A. moschatum* (Read and Hill 1988). Perry and Knowles (1991) suggest that stand regeneration, which is primarily by gap filling, may have generated genotypic structure as a result of clustered cohorts of similar genotypes with the resulting genotypes being structured by age. Since *A. moschatum* stands were shown to exhibit a variety of stand size structures and regeneration patterns (Chapter 4), yet had consistent genetic spatial structures, genetically differentiated regenerating cohorts seem an unlikely cause of genetic structure in this case.

It takes several generations for family structure to develop (Turner *et al.* 1982, Sokal and Wartenberg 1983, Epperson 1989). Knowles *et al.* (1992) interpreted the

differences in presence of genetic structure in populations of *Larix laricina* as reflecting differences in stand origin. They postulated that structure had developed where a few remaining trees (bottleneck) formed the basis for the present population, whereas the stand originating from heterogeneous outside seed sources had not yet developed a family structure. Coates (1992) however found no substructuring in populations of *Stylidium coroniforme* recovering from severe bottlenecks. Selection against selfed offspring or in favour of heterozygotes can counter the effects of inbreeding and lead to greater proportions of heterozygotes than expected and randomisation of genetic structure (Xie and Knowles 1991, Coates 1992, Ledig 1986, Epperson 1989). There was no evidence for heterosis in *A. moschatum* populations (Chapter 4).

As the *A. moschatum* stands are likely to have different histories, the results suggest that all have been stable long enough for some family substructuring to have developed. Stand size reduction, or bottlenecks, through time are likely to have facilitated substructuring. To the same extent founders of more isolated populations, in the absence of extensive seed rain, may have facilitated substructuring and genetic composition in those stands. Given the longevity of the species one could predict all the stands studied could be at least several hundred years old.

Therefore these results provide strong evidence that within *A. moschatum* stands family clusters of related genotypes are formed due to vegetative reproduction and localised pollen and seed dispersal. This would result in inbreeding within clusters as recorded in the previous chapter. Within stands there may be high allelic diversity, but little mixing of genotypes due to substructuring, and hence leading to the fixation of genotypic proportions. One could predict that through this mechanism localised fixation and differentiation of allelic frequencies could develop within larger population ensembles, such as was found to have occurred between the Frodshams and Creepy Crawly sites without the need for selection as a driving force. These results suggest that genotype distribution and frequencies within stands may largely reflect chance and historical occurrences. It is likely therefore that much variation in allelic proportions between many *A. moschatum* stands may be due to random events and drift.

The results have implications for conservation management. These include the acknowledgement that if there are small neighbourhood sizes in *A. moschatum* stands then, stands should not be considered too small to be worth conserving, since they mostly interbreed on a local scale, and even the smallest stands in this study have been relatively stable. Furthermore, large semicontinuous populations are unlikely to

be homogenous. Therefore it cannot be assumed that conserving one part of a large population will reflect or adequately sample the genetic variability contained in the whole, since local differentiation or drift, has occurred in *A. moschatum* populations.

GENERAL DISCUSSION :

Regeneration

Both Huon pine and sassafras tend to occur in small mixed-aged stands. This is in contrast to the large even aged stands reported for several New Zealand gymnosperm trees (Ogden 1985, Norton *et al.* 1989, Stewart and Rose 1989). The stand structures of both species were variable between sites (Figures 2.2 & 4.2), the range being consistent with the results of Barker (1992) for *Phyllocladus aspleniifolius*. This indicates some variability in regeneration depending on site conditions as has been noted in other species by several authors (Veblen 1989, Spies and Franklin 1989, Canham 1989). While the results suggest that both species respond to minor canopy openings with increased regeneration, neither appear to require large scale exogenous disturbance to maintain stands and persist. This contrasts with the mass regeneration following large-scale disturbance which is reported to be a common regeneration strategy in many New Zealand tree species (Ogden 1985, Stewart and Rose 1989). Since both Huon pine and sassafras can regenerate beneath their own canopies they can be classified as climax species by Whitmore's definition (1989). This is also consistent with the results of Read (1985) which found these species to have shade tolerant juveniles. However, both species have small, potentially well dispersed seed, produced in large quantities. This is a feature not associated with shade tolerant climax species, and therefore these species may be expected to behave as species at the least shade tolerant or pioneer end of the shade tolerant continuum (Whitmore 1989, Canham 1989). The survival of germinants appears to be a major determinant of the proportion of young saplings found in populations of both species. This has also been suggested to be the case in *Phyllocladus aspleniifolius* (Barker 1992). Since both species are long-lived, only infrequent replacement within a stand is necessary for maintenance (Levin 1978). Some stands of both species appear to have been persistent for very long periods in the absence of fire (Neyland 1991, Francey *et. al.* 1984, Gibson 1986, Anker *et al.* 1993). Regeneration is therefore likely to be adequate for long term survival of stands.

Sexual reproduction is clearly an important part of the regeneration process in each species. However there may be a high energy cost associated with sexual reproduction (Sedgely and Griffin 1989, Ellstrand and Antonovics 1985). Huon pine stands with higher proportions of cone bearing trees were associated with stand structures which would allow greater light penetration of their canopies (Chapter 2).

It was also found that as Huon pine trees got bigger they were more likely to produce cones (Figure 2.3). Mature canopy height trees may have greater potential for energy production due to more favourable conditions for photosynthetic activity and less energy required for growth (Bell 1985). They are therefore in a better position in terms of energy costs to direct energy into sexual reproduction (Linhart and Mitton 1985).

Both species produce prolific quantities of seed (Hickey *et al.* 1982). However, they clearly also utilise vegetative reproduction to persist on some sites. The Mt. Read site for Huon pine may be an extreme example. This site appears to be derived principally from a single clone and to date no females have been recorded (this study and pers. comm. M. Peterson). Monoclonal populations are unusual in clonally propagating species (Ellstrand and Roose 1987). Huon pine on this site has been dated back at least 11000 years from wood material (Barbetti *et al.* 1993). However, this may not amount to many generations, and if as Lynch and Gabriel predict, clonally derived populations can survive up to 10^4 generations then this site may have a long lifespan yet! The consistent spatial genetic structure found within stands in both species (Chapters 3 & 5), imply that vegetative reproduction is a universal feature of the regeneration of these species. The results of this study lend weight to the observations that clonal spread occurs in some temperate forest trees (Whitmore 1989), and is particularly common in Australian temperate rainforest trees (Johnson and Lacey 1983). Vegetative reproduction in these species is in the form of basal sprouts, layering and suckering. It bypasses the vulnerable germinant seedling stage, may allow more rapid growth, and may confer sexual maturity at smaller sizes. The degree of vegetative reproduction appears to vary between sites in both species. There may be evidence however of selection for or against vegetative reproduction in some sassafras populations. For example vegetative reproduction is characteristic of all Tasmanian sassafras populations, whereas it is much less evident in Victorian sites, and vegetative reproduction was not observed at all in the morphologically distinctive New South Wales sites.

As pointed out by other authors, it is often difficult to distinguish between vegetative sprouts and seedlings once they pass a certain size (Schnabel *et al.* 1991). Schnabel *et al.* (1991) pointed out in their study that many of the protected environments suitable for seedling survival are also sites where vegetative reproduction is also more likely (e.g. along logs and at the bases of trees). Such is likely to be the case in both the species in this study. Further many of the conditions which facilitate or induce vegetative reproduction, such as tree fall, may, by opening the canopy, stimulate sexual reproduction in adjacent trees, enhance germination and stimulate

growth in seedlings and saplings (Whitmore 1989, Spies and Franklin 1989). Thus both responses may enable these species to act more rapidly to colonise or fill canopy gaps. The results of this study tend to suggest that both species are fairly opportunistic in their regeneration responses. In these species it appears that both sexual and vegetative reproduction are utilised to maximise their chances of survival and persistence.

Huon pine and sassafras are interesting as they have qualities characteristic of both R and K strategies. For example their longevity is more typical of a K strategist but their prolific production of small seeds is a characteristic R feature (MacArthur and Wilson 1967). Vegetative reproduction may be more akin to a K strategy for reproduction since each shoot has a greater energy resource and therefore a greater chance of survival. Generally Tasmanian rainforest trees species seem to have these kinds of features. They are long-lived and most produce small seeds prolifically though not necessarily annually (Hickey *et al.* 1982), and most utilise vegetative reproduction to varying degrees (Cullen 1987, Read 1985). However, *Phyllocladus asplenifolius* seldom utilises vegetative reproduction, has more moderate seed production and is apparently highly successfully bird dispersed (Barker 1992).

Regeneration After Perturbations and Colonising Potential

Infrequent rainforest fires have been part of Tasmania's history (Barker 1991). Huon pine is killed by fire (Gibson 1986), whereas sassafras is capable of resprouting (Barker 1991). As both species produce large quantities of seed which may be capable of long distance dispersal, burnt sites could potentially be recolonised from neighbouring seed sources, initially from soil stored seed, or in sassafras by resprouting (Barker 1991, Schupp *et al.* 1989). Seedling regeneration has been observed in sassafras sites following fire (Barker 1991, Hill 1982). However, Neyland (1991) speculated incoming seed rather than soil stored seed to be the major source for regeneration in the small east coast sassafras populations. The relatively high genetic diversity observed in east coast sites may support this theory (Chapter 4). Recolonisation by seed is limited however in Huon pine populations, as dispersal is either highly localised or is limited to the floodline (Chapter 1). Huon pine seed may infrequently be dispersed long distances by green rosellas (Chapter 1), and this is the most likely explanation for some of the origins of high altitude stands, but is unlikely to be a major source of incoming seed to sites. In this species stands in the upper headwaters of river catchments or stands away from rivers banks, arguably the populations most fire prone, are unlikely to receive seed from neighbouring populations. Such populations are therefore more prone to local extinction. There are

many species like Huon pine whose seed dispersal is largely limited to down stream water flow (Schneider and Sharitz 1986). For these types of species the potential for recolonisation of sites by incoming seed following major disturbance will be limited, depending on the location of the populations relative to water courses, and if there are other populations upstream. If the flow is interfered with or diverted, populations may become more isolated from incoming seed and therefore more vulnerable to local extinction. In the case of species with restricted distributions this may be critical. For both these species therefore, the proximity of neighbouring stands may hold the key for the re-establishment of sites after major perturbations such as fire, although sassafras may resprout after low intensity fires (Barker 1991)

Huon pine seed seems to require very moist to wet conditions for germination and new germinant seedlings may be prone to desiccation. Such constantly moist conditions may not often be met in recently disturbed sites. Hence Huon pine is unlikely to be successful as a primary coloniser, but may be able to establish once a ground cover has been established. Due to their slow growth germinants can easily be smothered by other plants including mosses and lichens (pers. obs.). However, once established, seedlings may persist sheltered from desiccation underneath faster growing regrowth. Gibson (1986) found Huon pine seedlings recolonising burnt sites adjacent to live forest growing amongst other regrowth. Survival of sprouts and seedlings is critical for regeneration or recolonisation. Barker (1991) found that both seedlings and resprouts of sassafras were heavily browsed in a site regenerating after fire. Neyland (1991) also found heavy browsing of sassafras seedlings. Therefore sassafras seedlings are likely to survive only where hidden from herbivores. Many authors have suggested that one of the reasons seedlings of many species survive, or are observed growing on mounds, logs, etc., is due to their providing sites less susceptible to herbivory (Barker 1992, Harman and Franklin 1989). Vegetative sprouts may have advantages over seedlings by being able to reach larger, 'safe' sizes more rapidly (Johnson and Lacey 1983).

In these species the recruitment or recolonisation stage is likely to take up to several hundreds of years if recolonisation is predominantly by incoming seed. Such regeneration is more likely to lead to the mixed aged, and genetically variable stands observed in these species Neyland (1991). Long recruitment phases have been predicted for some *P. aspleniifolius* populations in Tasmania and for some long-lived New Zealand temperate trees species (Barker 1992, Norton *et al.* 1988). Such regeneration however, is in contrast with the mass regeneration observed in some New Zealand species (Ogden 1985), and may reflect a different history of disturbance to which New Zealand and Tasmanian species have become

differentially adapted (Denslow 1980). Dispersal and colonising potential will affect the rate of expansion of populations of both species. Evidence of active expansion was mostly observed fringing creeks in Huon pine (Chapter 1), but expansion at the edges of existing stands was more widespread in sassafras (pers. obs.). Sassafras is clearly more flexible in its ability to survive perturbations and recolonise sites than Huon pine. The widespread distribution of sassafras compared to the restricted range of Huon pine may reflect its greater dispersal and colonisation potential.

Density

Most Huon pine and sassafras stands were of similar density (Tables 2.4 & 4.5) and both species grow to trees of roughly similar dimensions. This may indicate some basic similarities in density determining factors operating in both species in the rainforest habitat (Antonovics and Levin 1980). Huon pine stand density was more variable than that of sassafras, with some much more dense stands recorded (Table 2.4). The Wentworth Falls sassafras stand was the only one in that species of comparable density to the most dense Huon pine sites. The sassafras at this site had a different growth form and it was a very unusual exposed site. The balance between sexual and asexual reproduction has been shown to be affected by density in some species, with asexual reproduction increased with higher densities (Abrahamson 1975, Hill 1973, Ogden 1974). While this may or may not be so in Huon pine, the most dense sassafras site differed from most others in there being no evidence of vegetative reproduction. The size of these plants was much smaller than in all other sites. This may suggest some density dependent regulation of plant size at this site, rather than the decreased size of plants being solely due to site factors such as decreased soil fertility (Antonovics and Levin 1980). The density of sassafras stands was not geographically correlated (Table 4.14) which contrasts to the pattern of density in Huon pine sites (Table 2.3). In Huon pine density was also weakly correlated with climate and the most dense stands were located in the south east of the Huon pine distribution (Chapter 2). These stands are in drier more marginal gallery rainforest habitat and may be expected to encounter disturbance from fire more frequently. Huon pine trees in these sites tend to be smaller, which may indicate a faster turnover rate of trees. The least dense sites had more older/larger trees. These results suggest that the self thinning rule (Harper 1977) applies to some extent in Huon pine populations. The alluvial soils on which most Huon pine populations are found is likely to be relatively fertile, however soils in the eastern sites are likely to be more fertile than those in the west due to the differing parent rock types (Jarman *et al.* 1991, Gibson *et al.* 1991). The eastern sites may also therefore be capable of sustaining a greater standing crop. Sassafras populations

cover a much wider environmental spectrum, yet a narrower range of differences was found in sassafras densities within stands. Therefore density seems to be less affected by site conditions in this species. It may also reflect the shorter lifespan of sassafras trees relative to Huon pine and relative to the frequency of perturbations experienced. The mainland sassafras populations were the most differing in their densities. This may in part reflect quite different environmental pressures and disturbance cycles at these sites than are found in Tasmanian sites. Such differences may also be reflected by the association between the density and genetic similarity of sassafras sites, as the most genetically distinctive sites were also on the mainland (Table 4.14). There was no corresponding trend between Huon pine site densities and genetics (Table 3.12).

Reproduction Modes and Inbreeding

Plant density has been predicted to have an affect on levels of inbreeding in plants (Antonovics and Levin 1980, Farris and Mitton 1984). The results of this study may therefore have been expected to show variation in the levels of inbreeding with density, and to reflect different trends between the two species with density due to their differing pollination mechanisms. It has been postulated that higher plant densities in insect pollinated plants, such as sassafras, will result in higher levels of inbreeding, as pollinator flights will be shorter, restricting gene flow (Farris and Mitton 1984). Antonovics and Levin (1980) however, predicted and reviewed evidence to suggest that in insect pollinated plants, declining density would lead to increased inbreeding. Whereas in wind pollinated species, such as Huon pine, Farris and Mitton (1984) suggested an individual in a high density population will be in contact with a pollen cloud containing a relatively small proportion of its own pollen, promoting outcrossing. This may not be so for dioecious trees, but if related trees are neighbours then related pollen will dominate the pollen arriving at any tree in less dense populations. However, in neither species was there any indication of an association between variation in density and variation in amount of inbreeding (e.g. Table 3.11), though both species were generally inbred. There was no indication of any differing trends in the effects of density on inbreeding between pollination mechanisms in these species. These results suggest that in these species the density of trees is not the determining factor affecting the level of inbreeding.

Both vegetative reproduction and localised dispersal are predicted to lead to the formation of clusters of related individuals, which are more likely to mate with each other (Epperson 1989) leading to biparental inbreeding (Brown 1989). In each of these two species the deficiency of heterozygotes and high level of inbreeding will

have occurred as a result of biparental inbreeding and vegetative reproduction with some selfing in sassafras (Chapters 3 & 4). The monoecious nature of sassafras may have contributed to the slightly larger scale of structure observed in that species. The extent of localised substructuring observed in both species was fairly consistent with substructuring observed in several other tree species (Schnabel *et al.* 1991, Perry and Knowles 1991). Insect pollinated species are predicted to be likely to exhibit family substructuring and higher levels of inbreeding (Loveless and Hamrick 1984), the sassafras results (Chapter 5) are therefore consistent with this prediction. In Huon pine the genetic structure found was more localised and was probably mostly dictated by vegetative processes (Chapter 3). The dominance of a few individuals within stands is also likely to have contributed to the inbreeding observed. The uniformity of substructuring among sites in both species also suggests that the mechanisms producing the substructuring are a function of the biology of each species rather than dependent on site specific conditions.

Lande and Schemske (1985) have suggested that inbreeding and outcrossing are both stable modes in a continum. They suggested that if species are primarily outcrossing then selfing is more likely to result in inbreeding depression, and conversely, outcrossing in primarily selfing species is more likely to result in outbreeding depression. As both species are outcrossers then it may be expected by Lande and Schemske's (1985) hypothesis that inbreeding would result in less fit individuals in both species and perhaps therefore expect some evidence of selection against inbred individuals. Some studies have found evidence of selection against inbred individuals which is often manifested as selection favouring heterozygotes (e.g. Coates 1992, Farris and Mitton 1984, Eguiarte *et al.* 1992). Farris and Mitton (1984), Eguiarte *et al.* (1992) and Brown (1989) found that the proportion of heterozygotes may increase with age as they are preferentially selected over homozygotes at progressive stages in their life cycle. There was no evidence of this in either Huon pine or sassafras (e.g. Figure 3.5). This may suggest that inbreeding may not result in less fit individuals these species. Brown (1989) has noted that a paradox in expectations is often observed in plant species, whereby outbreeding plants often show less heterozygosity than expected and inbreeders show more. Given that Huon pine is mostly dioecious and therefore predominantly an outcrosser and sassafras being monoecious or dioecious at least partially so, the heterozygote deficiencies observed are consistent with the paradox outlined by Brown (1989).

It is uncertain if either of the species in this study displays inbreeding depression. However, the prolific seed production often observed in both species, together with the lack of evidence for selection favouring heterozygotes suggests that these species

do not suffer inbreeding depression, and if they do, it is so common as to have become the norm. Inbreeding depression is thought to be the cumulative result of deleterious alleles (Lynch and Gabriel 1990, Charlesworth *et al.* 1990). Lande and Barrowclough (1987) have said that populations can be purged of deleterious alleles by undergoing a series of bottlenecks or remaining in small populations. Lande and Schemske (1985) have also suggested that species with a long history of occasional population bottlenecks may be expected to show relatively little inbreeding depression. Conversely, species with large outcrossing populations may be expected to show substantial inbreeding depression when in small populations, and for mechanisms to prevent inbreeding, such as those leading to self-incompatibility, to be selected for (Charlesworth *et al.* 1990, Lande and Schemske 1985). Such a scenario whereby deleterious alleles are eliminated by a series of bottlenecks may explain the results in these two species as both are likely to have gone through bottlenecks during past glaciation cycles (MacPhail 1979). Populations are likely to have remained small for long periods, and as these species have such long generation times, neither species would be expected to have fully regained genetic diversity following the population bottlenecks which are likely to have occured during the Last Glacial (Whitlock and MacCauley 1990). The high levels of inbreeding observed in these two species however, contrasts with the reports for long-lived New Zealand trees in which populations were found to be more or less in Hardy-Weinberg equilibrium (Hasse 1992a &b, Hawkins and Sweet 1989).

Vegetative reproduction has been a feature of both species with multiclonal stands being the most usual formation. This is comparable with the population structure of many other species capable of clonal propagation (Ellstrand and Roose 1987). To some extent sassafras and Huon pine populations could be thought of as mostly acting as assemblages of large individuals rather than genetically interacting units (Ellstrand and Roose 1987), since there appears to be little breeding between genotypes (Chapters 3 &4). The Mt. Read Huon pine population was an exception to the multiclonal model (Ellstrand and Roose 1987), and perhaps is an extreme version of the concept of populations acting as assemblages of large individuals. This population appears to be principally derived from a single clone. It highlights the potential for persistence of individual genotypes and indicates that in Huon pine, small populations can and have, maintained themselves for extended periods without sexual reproduction. Lynch and Gabriel (1990) have predicted, that clonal lineages are unlikely to last more than 10^4- 10^5 generations. In this species, this may amount to persistence for a very long time! Despite obvious active vegetative reproduction a large proportion of trees in this stand also produce male cones. If this population had been derived from a female its history may have been much different as females

would have been likely to receive pollen from outside sources and produced some sexually derived offspring.

An increased proportion of heterozygotes with age in populations would also be indicative of selection favouring the progeny of sexual reproduction (Ellstrand and Antonovics 1985). Thus there was little evidence in this study that selection has favoured sexually produced offspring in either species. Ellstrand and Antonovics (1985) have suggested that where it is advantageous to be in minority, for example where the population is afflicted by pathogens or predators, sexually derived offspring are likely to be favoured as variants of the norm, whereas under conditions of strong mutualistic interactions, asexuality may be favoured. Neither species have a known history of disease related mortality as do some other Tasmanian tree species such as *Nothofagus cunninghamii* (Packham 1991) and *Acacia dealbata*. Indeed Huon pine has been noted for its lack of disease related mortality, although occasional instances do occur (Davies 1983). The most noticeable causes of mortality in sassafras seem to be associated with disturbance (Neyland 1991). There is little evidence therefore, that there would be strong selection favouring sexual reproduction in these species, whereas selection for maintaining successful genotypes may be strong. This may have contributed to the balance between the levels of vegetative and sexual reproduction seen in both species (Antonovics and Levin 1980).

Diversity

Dioecious and monoecious species, wind pollinated species, and species with long distance seed dispersal, have been found to generally have lower levels of diversity among populations and to have most variation within populations (Hamrick and Godt 1989). Therefore it may be expected that there may be low diversity among populations in both of these species. However, inbreeding and clonal reproduction within populations are generally thought to lead to greater differentiation among populations (Hamrick and Godt 1989, Ellstrand and Roose 1987, Brown 1989). Thus we may expect this to have increased the differentiation between sites in these species. In both species, even though stands were inbred, most diversity was found within rather than between sites with most of the allozymes recorded for each species present in most sites (Chapters 3 &4). Therefore the amount of diversity among sites in either species was generally low and like that found for other long-lived, temperate, late successional species (Hamrick and Godt 1989). The levels of diversity among sites in both species are generally comparable with those found in New Zealand tree species (Hasse 1992a &b, Hawkins and Sweet 1989, Billington

1991). Although sassafras is more genetically diverse than Huon pine, like the New Zealand species, neither sassafras nor Huon pine are overall genetically diverse (Hasse 1992, Hawkins and Sweet 1989). We may thus expect a fair level of similarity between sites, at least in Tasmania, as there is little overall allelic diversity. It has been suggested that the New Zealand species (Hasse 1992 a & b, Hawkins and Sweet 1989), have a history of past bottlenecks and subsequent expansions from common sources, and this may have resulted in the loss of rare and uncommon allozymes from many sites, and lead to the similarity between present sites. Such a scenario would also be relevant for Huon pine and sassafras populations and may explain the fairly low allelic diversity observed.

Insect pollinated species are generally expected to have greater differentiation among populations (Hamrick and Godt 1989). Sassafras is insect pollinated, whereas Huon pine is wind pollinated. The diversity among sassafras sites was greater than among Huon pine sites. This however, may also reflect the wider geographical and ecological range of this species including its occurrence on the Australian mainland. In sassafras, wind dispersal of seed is the major source of gene flow and may have contributed to a lowering of diversity among sites.

The relatively low levels of differentiation between most sites suggests, that some gene flow between populations does or has occurred. It is also clear from the high levels of inbreeding and genetic substructuring in stands, that most gene flow is localised, therefore long distance gene flow is probably relatively infrequent. Relatively little gene flow may be needed to maintain low population differentiation in the absence of selection (Ellstrand 1992). It has been predicted that as few as one individual per generation may be all the gene flow necessary to maintain allelic diversity in small populations (Lande and Barrowclough 1987). Given the long generation time of each of these species, successful long distance dispersal events need only be very rare. The persistence of individuals by vegetative reproduction may also delay the loss of some allozymes due to drift, and maintain the relative proportions of allozymes present in stands (Ellstrand 1992). Within Tasmania it is feasible to expect the possibility of rare dispersal. The mainland sassafras populations however, are now likely to be totally isolated from the Tasmanian ones, as would the central N.S.W. populations from the Victorian populations. These populations are therefore likely to become further differentiated with progressive time. The results of this study may therefore, be generally supportive of the predictions that only low levels of gene flow are required to maintain allelic diversity in populations (Lande and Barrowclough 1987, Ellstrand 1992).

Genetic differentiation among Huon pine sites was mostly site specific with few trends apparent other than the effects of isolation and an association with the proportion of females (Table 3.12). Thus the differentiation observed may reflect localised selection or the product of chance allelic frequencies. Genetic differentiation among sassafras sites was correlated with geography, climate, species composition and density (Table 4.15). Some sites located within two kilometres of each other (e.g. Creepy Crawly/Frodshams, and Mt. Field/ Mt. Field High) were found to have significantly different allozyme compositions. This, together with the correlations between genetic differentiation and ecological/environmental differences, suggests that selection may have played a more prominent role in differentiation among sassafras sites than in Huon pine populations. This result may to some extent also reflect the shorter lifespan and faster growth of sassafras trees relative to Huon pines.

Small Populations

It is generally expected that small populations will be more inbred than large ones (Lande and Barrowclough 1987, Brown 1989). It has also been predicted that small populations will eventually suffer loss of allozyme diversity and may be missing rare alleles or have them in unusually high proportions due to chance (Barrett and Husband 1989, Krusche and Geburek 1991). These results however, showed no consistent differences in stand structure, density, inbreeding, or allelic diversity between isolated small populations and large populations in either species. These results are therefore in contrast to these expectations. Whilst some individual small populations within both species were genetically depauperate, supporting the above hypothesis, the variation in the amount of genetic diversity in small populations was as great as the variation in the amount of genetic diversity observed amongst stands within larger assemblages of either species. Such results are comparable with the distribution of genetic diversity found by Coates (1988), Moran and Hopper (1987), and Billington (1991). Genetic structure within stands was consistent regardless of density or stand size (Chapters 3 & 5). This indicates that localised pollen and seed dispersal, and vegetative reproduction are more important than population size in determining the amount of inbreeding. In these species where small populations are relatively stable, population size is clearly not an important issue, such a conclusion was also reached by Billington (1991). However, this may in part be due to the long distance dispersal potential of these species (Govindaraju 1988). If that is so, then the density of small stands i.e. the distances between them, may be important for their long term viability (Lande and Barrowclough 1987). For example, stands probably need to be close enough together so that dispersal between populations occurs at a

high enough frequency to be effective in maintaining genetic diversity and to restock damaged populations (Lande and Barrowclough 1987). Both species however, indicate a strong ability to persist at sites by vegetative means and in Huon pine in particular, genetic diversity within populations has not been necessary for the survival of some individual stands (e.g. Mt. Read) for at least 11000 years (Barbetti *et al.* 1993).

The general effect of population subdivision is thought to be to increase the variation among subpopulations but decrease the variation within each subpopulation (Lande and Barrowclough 1987, Levin 1988). Local allelic fixation and differentiation of subpopulations is at the heart of Wright's (1978) shifting balance theory of evolution. Both species are often found as small stands in subdivided populations. In both species allelic fixation has occurred within sites (Chapters 3 & 4) and has lead to differentiation among sites, thus generally supporting this theory. Theoretical models have lead to postulations that subdivided populations can be considered approximately panmictic if separate stands exchange as few as one or more migrants with each other per generation (Lande and Barrowclough 1987). However, this is said to be less true in linear population structures (Lande and Barrowclough 1987). In Huon pine, stands are usually scattered linearly along river banks, whereas in sassafras, stands are scattered in more two dimensional arrays. Thus it may be expected that Huon pine sites may be more likely to become differentiated than sassafras subpopulations. Generally sassafras sites became more genetically distant with geographical distance (Table 4.15), whereas the similarity of Huon pine sites was not related to their geographic proximity (Table 3.12). To some extent the lack of a trend with geographical distance may be a result of the linear nature of Huon pine populations which would support the contention that sub populations in linear arrays are less likely to act as large panmictic units. This has implications for conservation management of Huon pine and similar such species which occur as a series of linearly arranged sub-populations, as gene flow between sub-populations is unlikely to be equal, and therefore some populations will be at greater risk of local extinction than others .

Geographical Distribution of Diversity

Generally allelic diversity was distributed throughout the distribution of each species rather than being localised into one or more centres of diversity. Such centres of diversity are often used to speculate as to the area of origin of present populations of species (e.g. Schwaegerle and Schaal 1979). No clear centres were found for either species, however one sassafras site (Murchison Highway), located in the north west

of Tasmania, was unusual in containing all the allelic forms of each enzyme loci found in Tasmania (Table 4.6). Is it coincidental that this site would have been in the general vicinity of a possible large refugial area for sassafras during the Last Glacial? The lack of clear centres of diversity may suggest that both species survived the Last Glacial in many scattered sites. The spread of allelic diversity also means that for adequate conservation of the genetic diversity in either species, populations from throughout the range of each species need to be conserved (Lande and Barrowclough 1987). Currently the short term survival of neither species is at risk. Therefore management for the long-term maintenance of genetic diversity, both within populations and within each species, to ensure their long-term survival, should be considered a major conservation priority for these species at present (Lande and Barrowclough 1987).

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APPENDIX 1 :

Study Sites.

LAGAROSTROBOS FRANKLINII SITES

Riveaux Creek (RIVE) (a tributary of the Picton River)

The site consisted of a narrow fringe along the creek abutted by regrowth from a clearfelling operation. Mature eucalypts were found up to the edge of the stand, suggesting that pre-European fires had burnt up to the edges of this stand. This site was used as a study site by Millington *et al.* (1979). The upper section of this stand was mapped, below the mapped section, the stand became more patchy and narrower. This site was used for seedfall, germination, and seed dispersal studies.

Farmhouse Creek (FARM) (a tributary of the Picton River)

The stand at this site formed a narrow fringe along the creek surrounded by tall wet sclerophyll forest. The size, sex and location of the samples taken were recorded.

Picton River (PICT)

The site studied on the Picton River was located above the Tahune Bridge on the eastern bank. It formed a narrow fringe along the river and was abutted by tall wet sclerophyll forest.

Anne River (ANNE) (a tributary of the Upper Huon River)

The site was intersected by the track to Lake Judd and consisted of trees on a very narrow river fringe. In parts the river was reticulate. Heathy, open sclerophyllous vegetation and *Leptospermum* with a thick shrub layer was intermingled with the Huon pines.

Maxwell River (MAX)

Samples were collected from trees along the river bank by B. Prince (PWH archaeology).

Condominium Creek (COND) (runs into part of the Huon River now flooded)

Most of the Huon pine stand was located on the northern bank of Condominium Creek, interspersed amongst rainforest vegetation. Some sections were vegetated with rainforest understorey and a *Leptospermum* canopy. The southern bank was vegetated with buttongrass and *Leptospermum* with occasional Huon pines. The Creek was reticulate in parts.

Huon River (HUON)

The section below Blakes Opening and above Tahune Reserve was surveyed and the Huon pine distribution was found to be highly discontinuous. Huon pine trees were found only in more sheltered locations. The sampled stand was located below an island which offered some protection to the stand. The Huon pine grew in a narrow fringe along the bank.

Tahune Reserve (TAHN) (on the Huon River)

This stand consisted of a narrow fringe of trees along the Huon River with wet sclerophyll forest abutting it.

Junction Creek (JUNC) (a tributary of the Huon River)

The stand was surrounded by buttongrass dominated heathland and fringed by *Leptospermum* riparian scrub. Most of the stand fringed the often reticulate creek, although it extended away from the creek on the northern side. There was evidence that the stand size had been decreased by fire. Transects undertaken were both parallel and perpendicular to the creekline.

Stanley River (STAN)

This site was located away from the main river channel. There was evidence of burnt areas from the Savage River fires on the northern boundary of the stand. The stand was discrete and most of it was surveyed.

Yellow Creek (YELL) (the most northern population of Huon pine)

Electrophoretic samples were collected by J. Hickey. The sex of trees was determined where possible and the relative location of sampled trees recorded.

Pieman River (PIE)

Several sites were sampled in this region as follows.

Whyte River Junction (W) The transect started 100 m up the Whyte River and then followed the Pieman towards Corrina until Huon pines were no longer found. The transect followed a walking track.

Yanns Reach Inland (Y) Was a distinct stand (probably part of Pedley *et al.*'s 1980 D4) on the river flat away from the riverbank.

Yanns Reach Riverine (A) Was a narrow stand fringing the bank, probably part of Pedley *et al.*'s (1980) D2. The stand was located opposite the Savage River confluence.

Corinna Creek (B) - This stand was not recorded on Pedley *et al.*'s (1980) map. The stand occurred as a narrow river fringe.

Inland (C) - A stand corresponding to Pedley *et al.*'s (1980) I1 was surveyed. There were geological survey lines cut through this stand which ran from a boggy drainage line and up a slope. It was distinct and the whole stand was surveyed.

Inland (D) - This stand corresponded to Pedley *et al.*'s (1980) I2. It had been heavily logged and few living Huon pine trees are left. While it is well away from water courses, it occurs in a moist crater (limestone sink?) high above the river. Geological survey lines riddled this stand.

Pieman Inland (I) - This was probably part of Pedley *et al.*'s D7 stand. It was a small grove of trees set away from the water course with a relatively open understorey.

Newall Creek (NEWL)

This site was located on a narrow creek and associated drainage channels. One transect ran parallel to the creek and another perpendicular to it. There was some site disturbance associated with survey lines. There was also evidence of an old rock wall, probably part of an old tramway, and probably some localised clearing.

Teepookana (TEEP)

This site was highly disturbed by past logging. It is not associated with creeks or rivers, but reticulate drainage channels are present. This area was identified by Gibson (1986) as containing trees with a slower than average growth rate. Several transects were undertaken at this site.

King River (KING)

This study site has since been cleared of Huon pine and is now flooded by the King Hydro-Electric Scheme. The stand formed a narrow fringe along the river and was patchy in size structure. There were some areas of sandy beach with large trees present. There was evidence of previous Huon pine cutting by craftwood collectors. Eucalypt forest abutted the stand.

Mt Read(MTRD) (a high altitude site)

Where the Huon pine occurred at this site it effectively excluded other species. The stand was associated with drainage lines running into Lake Johnson. There was much evidence of layering and the trees tended to be horizontal for a lot of their length. Fire had burnt King Billy-dominated forest up to the northwestern edge of the stand. The general area is unique for its vegetative diversity including a large number of gymnosperms. Subsequent to our collection, isolated Huon pine plants were found remaining in a higher altitude, burnt Huon pine stand. These plants had krumholtz form (M. Peterson pers. comm.) and were sampled for electrophoresis. This is the highest recorded altitude for Huon pine. This site is currently being used for dendrochronological research by Cook, Buckley, Peterson, and co-workers.

Gilbert Leitch Reserve (GILB) (on the Gordon River above the Gordon Dam)

A small stand fringing a creek was surveyed. The surrounding vegetation was wet sclerophyll.

Frenchmans Cap (FCAP) (a high altitude site)

At this site the Huon pine occurred as emergent clumps associated with drainage lines. The surrounding vegetation contained *Athrotaxis selaginoides* and *Nothofagus gunnii*-dominated alpine heath. This community was originally reported by Hickey and Felton (1988). Samples were collected in addition to those on the transect.

Lake Vera (VERA) (near but below the Frenchmans Cap site)

The stands at this site were studied by Gibson (1986). Most samples were taken at several sites around the lake in addition to those on a small transect. The sexes of sampled trees were determined where possible. The track around the lake was used as a baseline for collections.

Franklin River (FRAN)

Upper Franklin River

Samples were collected from two locations by I. Robertson, one from near Log Jam Rapid, which was the first occurrence of Huon pine after it intersects with the Collingwood River, and one from near Rafters Race Rapids.

Irenabys

Trees from both sides of the river were sampled and their sex determened. Trees were found both fringing the river and further in from the banks. The site is at the base of a deep rainforest fringed gorge.

Great Ravine

Samples were collected by J. Marsden-Smedley from the cauldron portage track.

Lower Franklin

Samples were collected by I. Robertson and J. Marsden-Smedley from several sites.
1 Ganymead Pool 2 Glen Calder 3 Priona Cave 4 Jane River entrance

Gordon River Mouth (GORM)

Samples were collected from three sites and their size and sex determined. The collections at Pine Landing and site 3 were from trees fringing the river. The collections at Barnes Rivulet were from a transect leading from the river along a drainage channel.

Gordon River (GORD)

River fringing trees were sampled from between Kinghorn Creek and Eagle Creek. The sex was determined for each sample where possible.

Sir John Falls (JFAL) (Gordon River)

Trees fringing the Gordon River and the creek below the falls were surveyed. A second site was located on a plateau above the river. A survey line perpendicular to the river was used as a baseline for transects. The plateau area was moist underfoot but not associated with water courses.

Eagle Creek Track (EAGL) (Gordon river)

The track was followed for 1 km until Huon pine trees were found. The track cut through a stand at that point and was used as a baseline for transects. Young saplings up to 2 m tall lined the edge of the track in the upper sections of the transect. The stand was not located near obvious water courses. The conspicuous presence of *Gahnia grandis* and *Restio tetraphyllus* suggested site disturbance had occurred. The canopy was broken, and the stand scattered in small clumps.

Birches Inlet (BINL)

Samples were collected from two sites nearby Hobbs Creek by P. Brown (PWH).

Denison River (DENS)

The survey was undertaken within the Truchanas Reserve. Transects perpendicular to and along the river were undertaken. The forest was dense up to the river. Further samples along this river were collected by the Department of Parks, Wildlife and Heritage archaeologists and these sites have been recorded.

Greystone Bluff (GREY)

This stand is in a large basin perched high above the Davey River. The Huon pine is found in association with braided drainage channels which flow into Badger Creek and then to the Davey River. The surveyed stand was part of a large population. The site was notable for the consistent size of the trees and by the limited presence of epicormic shoots on Huon pine trunks.

Pine Creek (PINE) (a tributary of the Davey River to the north of Greystone Bluff)

The stand was in a basin rimmed by a ridge. Pine Creek formed a border with buttongrass dominated heathland. Huon pine seedlings were found extending into the heathland. The site was associated with a major channel draining into the creek and was essentially perpendicular to the creek.

Spero River(SPER)

Samples were collected along the bank for nearly the full extent of the stand and the locations of samples were recorded. Two transects were undertaken through stands on river flats away from the river fringe. The area has been described by Gibson (1986). Where the Huon pine occurred on river flats it was not generally also found on the river fringe.

Old River (OLD)

Samples were collected from trees along the river bank by P. Brown (PWH).

ATHEROSPERMA MOSCHATUM SITES

Mt Field (MFS) (Tas)

The site was sampled along the nature trail 'Lyrebird Walk' which was used as the centre line for a belt transect. The site was callidendrous rainforest with an open parklike understorey. *A. moschatum* was codominant with *Nothofagus cunninghamii* and there were sparse, emergent eucalypts in the site. The site was part of a much larger rainforest population containing *A. moschatum*. The site has also been used for study by T.Olesen.

Mt Field High (MFH) (Tas)

This was a high altitude site which followed a small creek, set within a boulder scree below Lake Fenton. The *A. moschatum* stand was dissected by a road but mostly was located on the downhill side seeming to reach its upper limit above it. Almost all *A. moschatum* plants present were sampled. The trees became more stunted as altitude increased. This site was at the high altitude extreme of a larger population and not far from the other Mt Field site. The site has been used by other researchers as some trees were tagged.

Mt Wellington (MWS) (Tas)

The stand was located in a steep gully on the lower slopes of the mountain and accessed by a walking track. The entire stand was mapped and almost all *A. moschatum* plants sampled. The stand was callidendrous in structure, having an open understorey with a diverse fern community. However, it was surrounded by wet sclerophyll forest and fairly isolated from other *A. moschatum* populations. The site has been used for fungal collections by other workers and leaf lichen samples were collected here for Dr.G.Kantvilas.

Frodshams Pass (FS) (Tas)

This site was a little further down the road from the Creepy Crawly site and was a south east facing gully dominated by large old *N.cunninghamii* and *A. moschatum* trees in a classic callidendrous forest. The surrounding rainforest was mostly dominated by *Phyllocladus asplenifolius* which has been studied by Barker (1992).

Bun Hill (BHS) (Tas)

This site was on the edge of a clearfelled coup which had encroached on the *A. moschatum* stand. It was a small isolated stand and fringed on its uphill side by *Pomaderris apetela* dominated implicate forest. Within the stand itself, *A. moschatum* was codominant with very large *Pittosporum bicolor* trees. The understorey was quite open and with much fern growth. Almost all of the stand was mapped and sampled. This site has been used as a study site by Jarman *et al.* (1991) and included in the survey by Neyland (1991).

Little Florentine (LFS) (Tas)

This was quite an unusual site as both *A. moschatum* and *Phyllocladus asplenifolius* were codominant with *N.cunninghamii*. The forest was callidendrous, having quite an open understorey and simple structure. There were several canopy openings caused by the death of *N.cunninghamii* trees due to myrtle wilt. The stand was in reasonably close proximity to other stands of *A. moschatum*. The site was also being used for myrtle wilt studies by J. Packam, G. Kile and H. Elliott.

Creepy Crawly (CCS) (Tas)

This site was thamnic to callidendrous in structure, with several rainforest tree species co-occurring in the canopy. The 'Creepy Crawly' ' rainforest interpretation track was used as a base line for the belt transect. There were some canopy gaps due to tree fall or death of trees from myrtle wilt. The site was inset within a larger rainforest population. This location has been used by many other researchers including J. Read and R. Coy (1991).

Bruny Island (BIS) (Tas)

The track to Mt Mangana was used as a baseline for a belt transect. The forest was very patchy and partitioned into different vegetation types. The *A. moschatum* occurred mostly in little gullies or drainage lines. There was evidence of disturbance throughout the site. *A. moschatum* would dominate thamnic forest patches. There are no large nearby tracts of *A. moschatum,* but there are scattered small pockets throughout the general area. The site was included in the survey by Neyland (1991).

Meetus Falls (MUF) (Tas)

A. moschatum was found in a small grove above the falls, but most of the population grows below the falls associated with a south facing moist gully, with some trees continuing along fringing the creek . The surrounding vegetation graded from wet to dry sclerophyll forest as distance increased. The transect was undertaken below the falls, here the forest was thamnic in structure and not very tall. The wet sclerophyll trees species *Pomaderris apetala* and *Olearia argophyla* were codominant with *A. moschatum* within the stand. This site was included in the study by Neyland (1991).

Wyefield Rivulet (WRS) (Tas)

This isolated *A. moschatum* stand was located broadly in the vicinity of Meetus Falls, it is sited in a protected gully with a small rivulet running through it and surrounded by eucalypt forest. The stand was quite well delineated but there was evidence that it was slightly larger in the recent past. The structure was thamnic and *A. moschatum* dominated the canopy. Some *A. moschatum trees* had fallen over then sent vertical shoots in to the canopy gap. This site was surveyed by Neyland (1991).

Tahune Reserve (THS) Tas)

This site was a mixed species thamnic rainforest with many wet sclerophyll species also present. There were some disturbed areas where trees had fallen over. The track following the Huon river down stream was used as a base line, but most *A. moschatum* trees were located on the side away from the river. The site is surrounded by wet sclerophyll forest, but other patches containing *A. moschatum* are scattered throughout the area. The site was also used in the Huon pine study.

Newall Creek (NAS) (Tas)

This was a thamnic rainforest site with evidence of small scale disturbance and a patchy distribution of tree species. The site was unusual in its canopy species composition, with *Athrotaxis selaginoides, Lagarostrobos franklinii, P. aspleniifolus* in the canopy as well as *A. moschatum, N. cunninghamii* and *Eucryphia lucida.* The site was set amidst a larger area of rainforest containing *A. moschatum.* The transect ran parallel to the creek beginning below the NRCP rainforest viewing platform. The site was also used in the Huon pine study.

Anthony Road (ARS) (Tas)

The site consisted of thamnic to callidendrous rainforest. *A. moschatum* was codominant with *N. cunninghamii, E. lucida* and tree form *Anodopetalum biglandulosum.* this site however was surrounded by extensive rainforest in which *P. aspleniifolius* was often a canopy dominant. This site was in the vicinity of Read and Hill's (1988) Murchison study site.

Murchison Highway (MHS) (Tas)

The site was located approximately across from the turn off to the Cradle Mountain link road north of Tullah. The dominant vegetation was comprised of very tall *N. cunninghamii* overtopping a subcanopy of large *A. moschatum* trees. It was callidendrous forest with an open understorey and a diverse fern component. This stand is located within an extensive rainforest assemblage.

Milkshake Reserve (MRS) (Tas)

This site was a small area of thamnic rainforest containing principally, *A. moschatum, N. cunninghamii* and *Dicksonia antarctica* , overtopped in parts by tall eucalypts. The canopy was quite disturbed in parts. The site was surrounded by wet sclerophyll forest, but throughout the general area there are large tracts of rainforest containing *A. moschatum* as a minor species.

Balfour Track (BTS) (Tas)

The start of the track was used as a base line for collecting. The forest was open callidendrous with a sparse distribution of large trees. The canopy was dominated by *N. cunninghamii, E. lucida, A. moschatum and A. biglandulosum.* At this site *A. biglandulosum, A. moschatum* and *E. lucida* all had unusually large, broad leaves. The site was located in a larger area of rainforest containing *A. moschatum.*

Liffey Falls (LIF) (Tas)

The stand was mostly along creek flats in a deep rainforest gully. The transect ran parallel to the creek in the vicinity of the waterfalls. The site was dominated by *A. moschatum* and *N.cunninghamii* with an open understorey. The gully was surrounded by wet sclerophyll forest. There are other such stands throughout the Western Tiers.

Mt Donna Buang (DBS) (Vic)

This site was in a protected gully, situated on the downhill side of the road (Acheron Way) a little before the turn off to Mt Donna Buang. The trees were restricted to the gully and approximately 80% of the stand was sampled. *A. moschatum* was co-dominant with *N. cunninghamii* and *Dicksonia antarctica* was common in the understorey. Also present were *Pomaderris aspera, Olearia argophylla* and *Bedfordia arborescens*. The site was similar to Tasmanian *A. moschatum* forest in structure. Other isolated gullies containing *A. moschatum* are scattered throughout the general area.

Tarra-Bulga National Park (TBS) (Vic)

This small isolated stand was located in the Tarra valley closely associated with a creek and surrounded by tall eucalypt forest with an understorey dominated by *Dicksonia antarctica* (see Ashwell 1991). The stand was similar in structure and composition to Tasmanian stands. However, it differed since *A. moschatum, N. cunninghamii* and *Hedycarya angustifolia* were in the canopy, both *D. antarctica* and *Cyathea cunninghamii* were in the understorey and the liane *Parsonsia brownii* was present. This is reported to be the largest such stand in the park (Ashwell 1991) and most of it was sampled for this study. *A. moschatum* is restricted to small stream side patches within the area.

Errinundra plateau (ERS) (Vic)

The site was in the Kanuka creek flora reserve. The forest was thamnic to callidendrous in structure with *A. moschatum* codominant with *Elaeocarpus holopetalus* in the canopy. *Pittosporum bicolor, Tasmannia lanceolata, D. antarctica, Blechnum wattsii* and *Polystichum proliferum* were also present in the stand. The stand was located in a fairly large gully running downhill from the road. Much of the surrounding area was comprised of regrowth forest following clearfelling forestry operations. Errinundra Plateau contains the largest tracts of temperate rainforest containing *A. moschatum* on the mainland. This kind of forest is described in Floyd (1990).

Wentworth Falls (WFS) (N.S.W.)

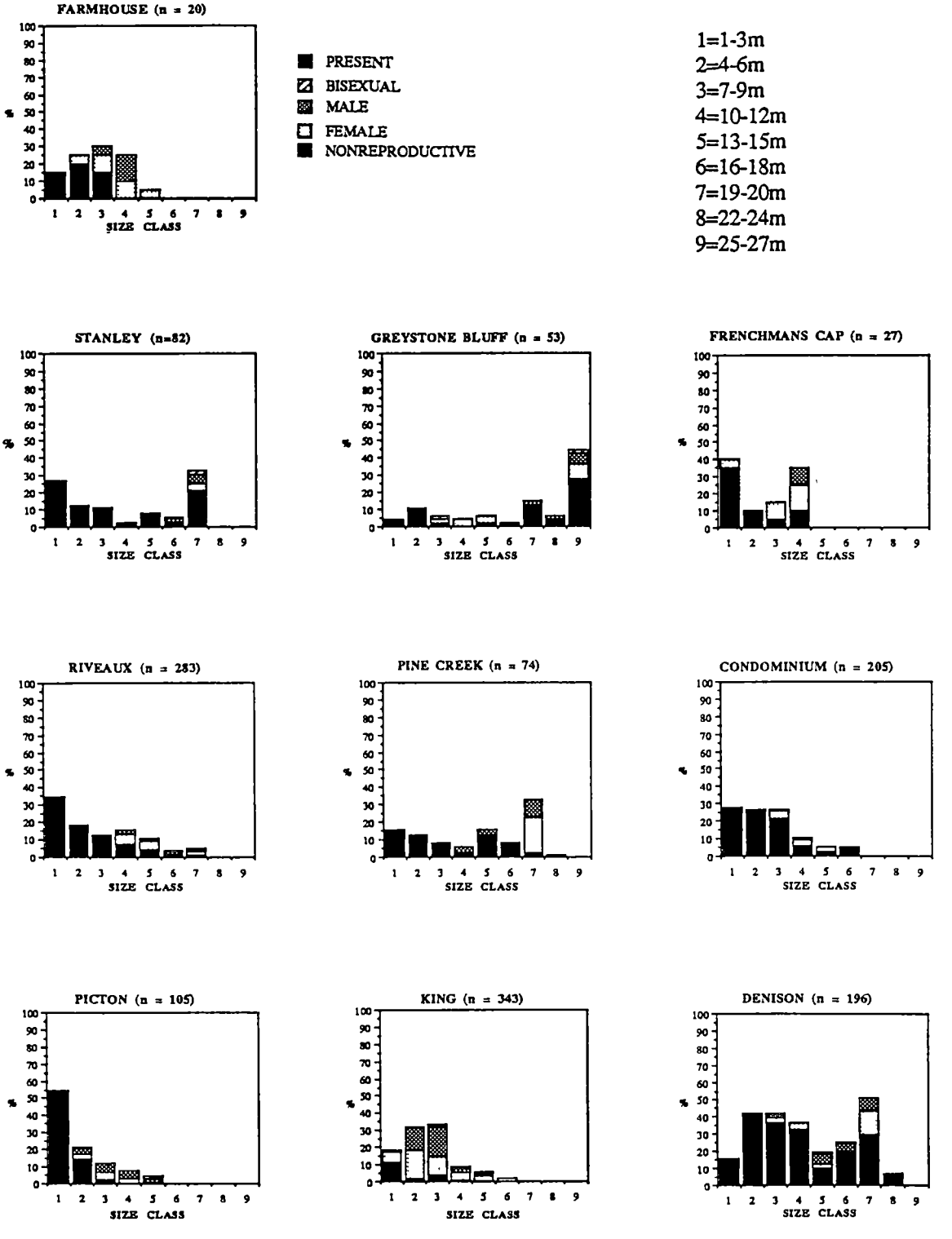
The stand was located in the spray zone of the major waterfall, along the edge of the vegetation at the base of the falls. Here *A. moschatum* grew as a fairly dense stand of shrubs mostly two to three metres tall, with diameters up to approximately five centimetres. The plants were often highly branched from the main stem but there was no evidence of basal sprouts or suckering. Leaves were reduced in width relative to length, and little evidence of serrations were present. Trees from approximately half the stand were sampled. *Microstrobos fitzgeraldii* was found growing in association in the more open part of the stand which otherwise was virtually monospecific. This is a very isolated outlying stand, however there are a few such stands within the Blue Mountains region. This population is reported in Floyd (1989).

Gloucester Falls (GFS) (N.S.W.)

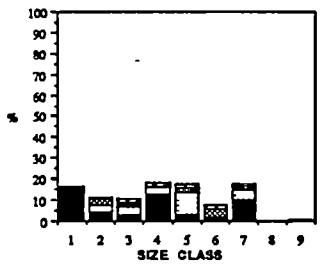
At this high altitude site, *A. moschatum* grows as a shrub to a small tree up to about ten metres height, with diameters up to fifteen centimetres and a slightly weeping form. The stand was mostly concentrated in a protected curve of forest facing the falls, but trees were also found fringing the creek further upstream. The surrounding forest was dominated by *Nothofagus moorei* and the vine *Smilax australis* was dominant in the understorey. Most of the trees in the stand were sampled. On the other side of the creek, the forest was subalpine eucalypt woodland. This is a very isolated outlying site, however there are several other known stands within the broad geographical region. The site is reported in Floyd (1990).

APPENDIX 2 :

Huon pine height distribution at the study sites



PIEMAN TOTAL (n = 125)

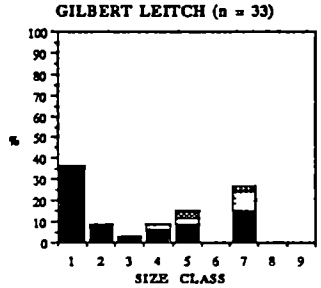


PRESENT
BISEXUAL
MALE
FEMALE
NONREPRODUCTIVE

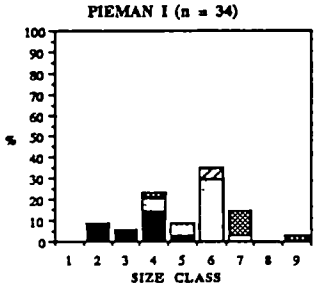
HEIGHT SIZE CLASS LEGEND:

1=1-3m
2=4-6m
3=7-9m
4=10-12m
5=13-15m
6=16-18m
7=19-20m
8=22-24m
9=25-27m

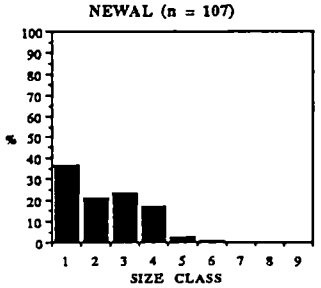
GILBERT LEITCH (n = 33)



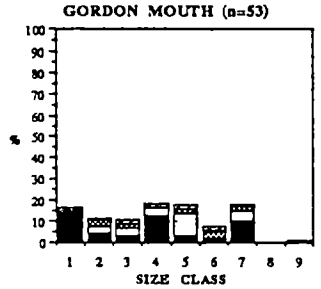
PIEMAN I (n = 34)



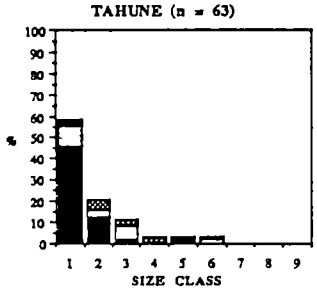
NEWAL (n = 107)



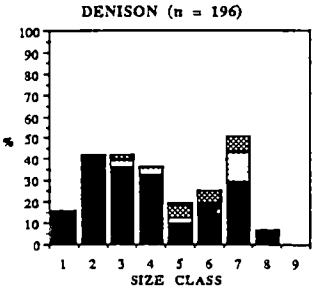
GORDON MOUTH (n=53)



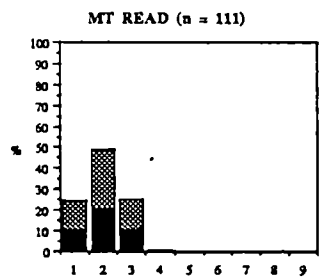
TAHUNE (n = 63)



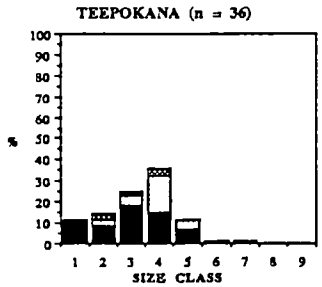
DENISON (n = 196)



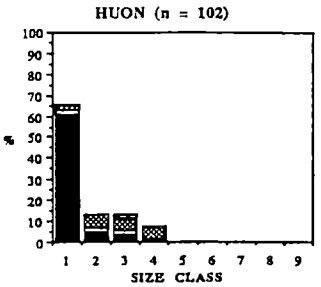
MT READ (n = 111)

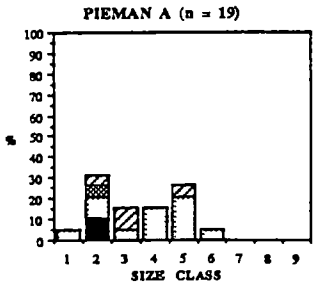


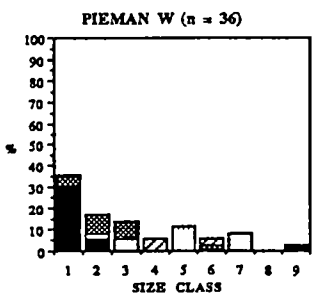
TEEPOKANA (n = 36)



HUON (n = 102)

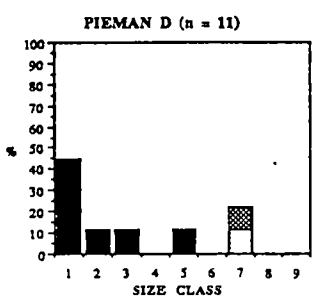


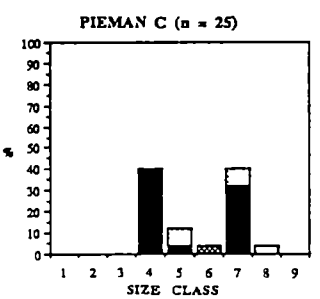


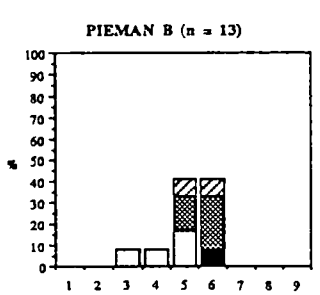


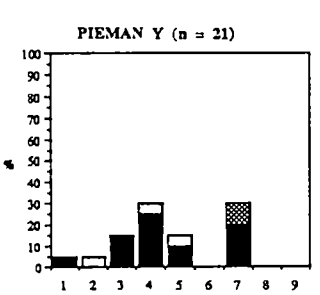
HEIGHT SIZE CLASS LEGEND:

1=1-3m
2=4-6m
3=7-9m
4=10-12m
5=13-15m
6=16-18m
7=19-20m
8=22-24m
9=25-27m









APPENDIX 3 :

Huon Pine genotypic frequencies.

Pop	IDH	6PG	G6PDH	AAT-1	AAT-2	AAT-3
Anne	14AA 13AC 6CC	1AA 32BB	2AA 31BB	33AA	33AA	33AA
Condaminium	17AA 18AC 20CC	3AA 52BB	7AA 5AB 43BB	55AA	55AA	55AA
Pine	11AA 12AC 17CC	3AA 37BB	10AA 1AB 29BB	40AA	40AA	40AA
Farmhouse	5AA 4AC 10CC	3AA 15BB 1CC	5AA 10BB 2BC 2CC	19AA	19AA	19AA
Franklin Upper	16AA 17BB 27CC	1AA 34BB 3CC	4AA 2AB 46BB 8CC	60AA	60AA	60AA
Greystone	10AA 12CC	1AA 21BB	1AB 18BB 3CC	22AA	22AA	22AA
Frenchmans Cap	6AA 6AC 9CC	21BB	21BB	21AA	21AA	21AA
Gordon Mouth	23AA 14AC 33CC	1AA 1AB 1AC 61BB 1BC 5CC	8AA 1AB 61BB	70AA	70AA	70AA
King	9AA 7AC 10CC	3AA 22BB 1CC	2AA 21BB 1BC 2CC	26AA	26AA	26AA
LVera	19AA 13AC 34CC	4AA 44BB 1CC	13AA 44BB 9CC	66AA	66AA	66AA

Mt. Read	42AA 4AC	45BB 1CC	5AA 41BB	46AA	46AA	46AA
Newall	45AA 7AC 5CC	10AA 2AB 38BB 1BC 6CC	12AA 3AB 41BB 1CC	57AA	57AA	57AA
Picton	20AA 1AC 18CC	39BB	34BB 1BC 4CC	39AA	39AA	39AA
Pieman	51AA 24AC 45CC	5AA 2AB 102BB 1BC 10CC	3AA 97BB 20CC	110AA	110AA	110AA
Riveaux	26AA 31AC 12CC	3AA 1AB 54BB 11CC	2AA 2AB 50BB 2BC 13CC	69AA	69AA	69AA
Spero	70AA 12AC 25CC	4AB 80BB 23CC	7AA 2AB 65BB 2BC 31CC	107AA	107AA	107AA
Stanley	20AA 5AC 8CC	33AA	2AA 1AB 30BB	33AA	33AA	33AA
Teepookana	52AA 5AC 14CC	7AA 62BB 2CC	2AB 58BB 2CC	71AA	71AA	71AA
Tahune	16AA 5AC 10CC	1AA 29BB 1CC	2AA 28BB 1CC	31AA	31AA	31AA
Denison	24AA 29AC 30CC	8AA 3AB 58BB 2BC 12CC	17AA 14AB 61BB 1CC	83AA	83AA	83AA
Yellow	6AA 1AC 2CC	9BB	4BB 1BC 4CC	9AA	9AA	9AA

Junction	14AA 6AC 17CC	1AA 35BB 1CC	2AA 1AB 29BB 1BC 4CC	37AA	37AA	37AA
Huon	6AA 3AC 12CC	21BB	20BB 1CC	21AA	21AA	21AA
Maxwell	3AC 2CC	5BB	5BB	5AA	5AA	5AA
Old	5AA 3AC 4CC	9BB 3CC	1AB 10BB 1CC	12AA	12AA	12AA
Birchs Inlet	5AA 9AC 16CC	16BB 1CC	3AA 1AB 20BB 2BC 4CC	30AA	30AA	30AA
Gilbert Leitch	9AA 5AC 11CC	1AB 11BB	5AA 20BB	25AA	25AA	25AA
Irenabyss	13AA 4AC 15CC	1AB 19BB 1BC 6CC	1AA 1AB 24BB 6CC	32AA	32AA	32AA
Sr. John Falls	9AA 6AC 26CC	2AA 1AC 17BB	3AA 2AB 33BB 3CC	41AA	41AA	41AA
Gordon River	37AA 10AC 54CC	2AA 2AB 7AC 49BB 2CC	19AA 2AB 68BB 3BC 9CC	101AA	101AA	101AA
Great Ravine	17AA 3AC 8CC	3AB 25BB	3AA 19BB 1BC 5CC	28AA	28AA	28AA
Franklin Lower	6AA 7AC 3CC	13BB 3CC	1AA 1AB 14BB	16AA	16AA	16AA

APPENDIX 4 :

Sassafras genotypic frequencies.

Population	AAT	GDH	6PG	SDH	PER	IDH-1	IDH-2	PGI-1	PGI-2	PGI-3
Mt. Field	1AB	13AA	39AA	11AA	35AA	39AA	39AA	39AA	26AA	14AA
	2AC	23BB		20BB	4BB				9BB	13BB
	36AA	3AB		7CC					4AB	11CC
				1AC						
Mt. Field High	21AA	10AA	12AA	16AA	20AA	24AA	24AA	24AA	11AA	2AA
	2AB	5BB	2BB	5CC	3BB				10BB	16BB
	1AC	9AB	10AB	3AC	1AB				3AB	2CC
										4AB
Mt Wellington	50AA	6AA	52AA	18AA	50AA	54AA	54AA	54AA	32AA	18AA
	2AB	43BB	1AB	18BB	1AB				16BB	18BB
	2AC	5AB		11CC	3BB				6AB	13CC
				1AC						5AB
Bun Hill	42AA	6AA	60AA	29AA	43AA	61AA	60AA	61AA	29AA	19AA
	5AB	29AB	1BB	11AC	9AB		1BB		11AB	12AB
	9AC	26BB		13CC	9BB				16BB	23BB
	2BB									2CC
	1BC									
	1CC									
Little Florentine	20AA	6AA	20AA	10AA	12AA	20AA	20AA	20AA	6AA	3AA
		4AB		2AC	2AB				1AB	1AB
		10BB			6BB				13BB	15BB
										1CC
Creepy Crawly	47AA	5AA	51AA	43AA	34AA	55AA	55AA	55AA	34AA	29AA
	5AB	8AB	4BB	10AC	10AB				4AB	5AB
	2BB	41BB		2CC	11BB				11BB	1AC
	1CC									2BB
										1BC
										11CC
Frodshams	32AA	16AA	35AA	11AA	16AA	35AA	35AA	35AA	13AA	19AA
	3AC	2AB		15AC	9AB				10AB	1AB
		16BB		9CC	10BB				7BB	2AC
		1CC								4BB
										4CC
Bruney Is.	21AA	6AA	26AA	9AA	17AA	26AA	26AA	26AA	12AA	12AA
	1AB	2AB		9AB	5AB				1AB	2AB
	1AC	18BB		4BB	4BB				13BB	12BB
	2BB									
	1CC									

Meetus Falls	44AA	16AA 10AB 18BB	44AA	16AC 19CC	22AA 8AB 14BB	44AA	44AA	44AA	25AA 11AB 8BB	27AA 1AB 12BB 2BD 2CC
Wyefield	37AA 7AB 5AC 2AD 7BB 1CC 2DD	22AA 20AB 19BB	60AA	30AA 1AB 18AC 3BB 8CC	28AA 14AB 18BB	60AA	60AA	60AA	28AA 8AB 23BB	29AA 7AB 20BB 1BD 2CC
Tahune	26AA 5AB 1AD 9BB	12AA 1AB 28BB	41AA	29AA 4AC 3BB 5CC	25AA 5AB 9BB 2CC	41AA	41AA	41AA	25AA 7AB 7BB	4AB 35BB
Donna Buang	35AA 9AD 2DD	18AA 3AB 2AC 11BB 12CC	46AA	29AA 5AB 4AC 7BB 1CC	21AA 5AB 20BB	38AA 1AC 3AD 4DD	32AA 9AC 4AD 1BB	44AA	20AA 14AB 12BB	24AA 6AB 13BB 3CC
Tarra Bulga	24AA 3AD	7AA 1AB 1BB 1BC 17CC	27AA	25AA 2AB	19AA 1AB 2AC 5BB	25AA 1AD 1CC	23AA 1AB 1AD 2DD	27AA	23AA 3AB 1BB	13AA 2AB 7BB 5CC
Errinundra	30AA 2AD	18AA 1AC 10BB 3CC	32AA	23AA 1AB 1AC 2BB 5CC	27AA 3AB 2BB	26AA 6BB	26AA 4AD 2DD	32AA	28AA 2AB 1BB	13AA 7BB 11CC
Wentworth Falls	24AA 7AD 5DD	36AA	36AA	21AA 1AB 3AC 4BB 1BC 6CC	20AA 4AB 11BB 1CC	28AA 3AB 5BB	29AA 1AC 4AD 2DD	36AA	32AA 4BB	11AA 4AB 2BB 1BC 18CC
Gloucester Falls	31AA 3AD 1DD	35AA	35AA	10AA 1AB 1AC 3BB 20CC	14AA 8AB 2AC 10BB 1CC	25AA 10CC	25AA 4AD 6BB	17AA 17BB	27AA 7BB	10AA 1AB 15BB 5BC 3CC

Newall	30AA 1AB 4AC 1CC	10AA 19AB 16BB	45AA	18AA 10AC 4BB 13CC	15AA 5AB 25BB	45AA	45AA	30AA 12BB	16AA 11AB 15BB	6AA 17AB 2AD 11BB 1BD 1CC 4DD
Anthony	23AA 8AC	10AA 17AB 4B	31AA	16AA 4AB 1BB 10CC	7AA 5AB 19BB	31AA	31AA	31AA	22AA 5AB 3BB	8AA 10AB 1AD 3BB 4DD
Murchison	48AA 2AB 1AC 3AD	19AA 18AB 15BB 2CC	50AA 2AB 2BB	15AA 15AC 16BB 8CC	30AA 7AB 17BB	54AA	54AA	37AA 1AB 15BB	35AA 6AB 12BB	22AA 10AB 2AD 9BB 1BD 2CC 7DD
Milkshake Reserve	50AA	18AA 18AB 14BB	50AA	12AA 12AC 8BB 1BC 17CC	20AA 7AB 22BB 1BC	50AA	50AA	33AA 1AB 16BB	30AA 9AB 11BB	13AA 12AB 5AD 6BB 4BD 1CC 9DD
Balfour Track	25AA 2AC 4AD	10AA 16AB 5BB	31AA	8AA 3AC 4BB 16CC	7AA 9AB 15BB	31AA	31AA	21A 3AB 7BB	25AA 6AB	5AA 8AB 1BB 6BD 3CC 8DD
Liffey Falls	44AA 3AB 1AC 4AD 1BD	10AA 21AB 22BB	52AA	20AA 8AC 1BB 24CC	25AA 8AB 20BB	52AA	52AA	47AA 5BB	30AA 9AB 13BB	13AA 11AB 9BB 9BD 1CC 9DD